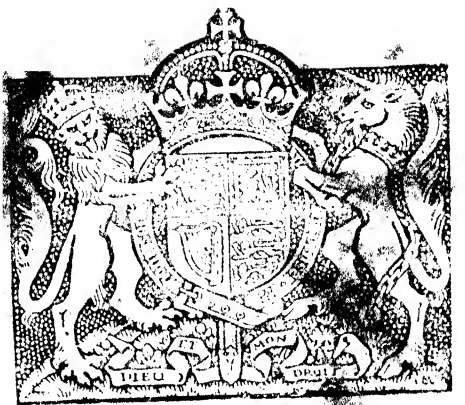


IMPERIAL AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI
19-2-47

M.G.I.P.C.—84—III I-23—22-8-45—5,000.

CANADIAN JOURNAL OF RESEARCH

VOLUME ~~XX~~ — XXI
1942–43

SECTION D

CANADA

19224
IARI

Published by the
NATIONAL
RESEARCH COUNCIL
of CANADA

19224

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. JANUARY, 1942 NUMBER 1

THE EFFECT OF TEMPERATURE ON THE CRITICAL OXYGEN PRESSURE FOR HEART-BEAT FREQUENCY IN EMBRYOS OF ATLANTIC SALMON AND SPECKLED TROUT[1]

BY KENNETH C. FISHER[2]

Abstract

Intact embryos of speckled trout and Atlantic salmon were exposed to solutions of oxygen and nitrogen in distilled water. It was observed that as the partial pressure of oxygen was reduced a pressure was found below which the frequency of the heart-beat was not maintained at the normal level characteristic of higher partial pressures of oxygen. A "critical" partial pressure of oxygen for heart-beat frequency can therefore be said to exist. Data have been obtained from which its value at five different temperatures can be determined. The critical pressures for the two organisms are similar, rising from approximately 3 to 5 mm. of mercury at 1.5° C. to 40 to 50 mm. of mercury at 20° C. Possible mechanisms leading to the establishment of a critical oxygen partial pressure are discussed. It seems likely that diffusion is not the limiting factor in these preparations so that the critical pressure, and its temperature coefficient, must be the property of the intracellular respiratory systems concerned. The finding that the logarithm of the critical partial pressure can be represented as a linear function of the reciprocal of the absolute temperature is consistent with this view. Temperature exercises a more pronounced effect on the critical oxygen pressure of the pacemaking process, than it does on the over-all velocity of that process as indicated by the normal frequency of the heart. It is concluded that the critical pressure is a characteristic of the chemical systems in the pacemaking cells of the heart.

Introduction

The oxidation mechanism in the pacemaking cells of the heart has recently been investigated in intact fish embryos by the use of carbon monoxide (2). An irregularity occurred in those experiments which was probably due to the low partial pressure of oxygen employed, rather than to the poisoning of some enzyme by carbon monoxide. It was thus necessary, before proceeding further with such experiments, to establish the partial pressure at which effects due only to oxygen lack might be expected to appear (i.e., to determine the *critical* pressure). In addition, since both Tang (10) and Kempner (5) have indicated that the critical oxygen partial pressure probably has a high temperature coefficient, it was desirable that the determinations be made at

[1] *Manuscript received July 26, 1941.*

Contribution from the Department of Zoology, University of Toronto, Toronto, Ont., with financial assistance from the National Research Council of Canada, granted through the National Committee on Fish Culture.

[2] *Assistant Professor of Physiological Zoology.*

19224

a series of temperatures. It is apparent that the temperature coefficient of the critical partial pressure of oxygen for the heart-beat frequency may itself serve to further characterize the chemical systems of the pacemaking process. Moreover, a knowledge of the critical pressure in fish embryos and its variation with temperature is important in ecological and cultural problems. Data concerning the critical partial pressure for the heart-beat frequency at several different temperatures in embryos of salmon and trout are reported in this paper.

Materials and Methods

Embryos of Atlantic salmon, *Salmo salar* Linnæus, and speckled trout, *Salvelinus fontinalis* Mitchill, were maintained in the laboratory in the manner described by Armstrong and Fisher (1). The organisms were used from a period approximately one month before they would naturally have hatched, until the increased pigmentation made observation of the heart difficult. If hatching had not yet occurred spontaneously, the chorion was removed a day or more before the organism was used for an experiment.

In each experiment the organisms were initially exposed to aerated distilled water. For subjection to various partial pressures of oxygen four embryos were held in a glass tube through which was maintained a flow of water (approximately 10 ml. per min.) containing oxygen at the desired partial pressure (2). Four such tubes were held in a rack immersed in a constant temperature water-bath so that the effects of four different partial pressures of oxygen were usually determined simultaneously. The results will be given as the average heart-beat frequency of the fish in each tube.

The constant temperature water-bath was cooled below room temperature by the continuous addition of cold water or by means of an electric refrigerating unit. Electric heaters controlled by a thermoregulator then raised the bath to the desired temperature. The sensitive element in the control circuit was a nickel resistance thermometer assembled for the purpose in this laboratory. The unbalance of the Wheatstone bridge containing the thermometer was used to operate a galvanometer relay which in turn adjusted the heating (cf. 7). Such a system is extremely convenient for rapidly and accurately shifting the temperature to predetermined values. With six volts across the bridge, temperatures were constant to $\pm 0.05°$ C.

Gas solutions containing oxygen at partial pressures from 150 mm. of mercury to approximately 10 mm. were prepared by saturating gas-free distilled water with oxygen–nitrogen mixtures. To obtain a continuous supply of water containing oxygen at pressures below 10 mm. of mercury, water was siphoned out of a flask that was kept at a constant level, and that was electrically heated at a constant rate (Fig. 1). The oxygen concentration in the heated water was made high or low by varying the rate of inflow and outflow. Since the solutions were rendered toxic by passage through tin, lead, copper, or aluminum tubing it was necessary to use the more fragile glass tubing throughout for cooling coils, etc. Rubber, though used at certain

joints where flexibility was required, was kept to a minimum since at the lower partial pressures appreciable quantities of oxygen pass through the wall of ordinary rubber tubing from the atmosphere. In some experiments this permeability of rubber to oxygen was employed with advantage to secure low pressures of oxygen.

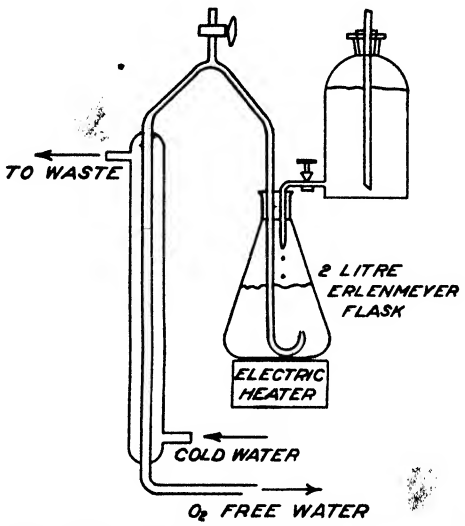


FIG. 1. *Apparatus which produces continuously water with a low oxygen content.*

After passage through a tube of embryos, the irrigating solution was led by means of a capillary to the bottom of a 10 ml. specific gravity bottle. The overflow passed to waste as drops, the frequency of which was a measure of the rate of flow past the embryos. As desired, the specific gravity bottles, filled to overflowing, were removed for analysis of the water for oxygen and a new bottle was immediately placed in position. On the average between four and five analyses were made on the gas solution passing over each set of four organisms, the samples being taken at intervals throughout the experiment. The variability of the partial pressure during an experiment was within the limits of reproducibility of duplicate analyses. In discussing the results therefore, the average of the analyses will be reported.

As stated by Lund (6) it was found also in this work that the Winkler method for the determination of dissolved oxygen can be adapted for use on a 10 ml. sample by employing smaller quantities of the reagents than are used in the standard procedure. Analyses were performed by the Winkler method as described by Sutton (9) but modified for water samples of 10 ml. by using only 0.05 ml. of the manganous chloride, 0.15 ml. of the alkaline iodide, and 0.2 ml. of the concentrated hydrochloric acid. The quantities of oxygen actually determined in this research varied for the most part between 2×10^{-5} and 2×10^{-6} gm. In this range the average difference between duplicate

analyses appeared to be an absolute rather than a relative quantity. As the method was employed it corresponded to approximately $\pm 4 \times 10^{-7}$ gm.

The partial pressures were calculated from the analytically measured quantities (M) of dissolved oxygen by the formula

$$p.p.O_2 = 760 \frac{M}{q}$$

q being the amount of oxygen dissolved in water at the temperature of the experiment when its partial pressure is 760 mm. of mercury (values of q were obtained from data given in the Handbook of Chemistry and Physics, 19th ed., 1935, Chemical Rubber Publishing Co.).

Results

During the preliminary period of exposure to aerated distilled water, the frequency of the heart-beats often showed a tendency to drift slightly but after the first hour or two became quite constant (the average deviation of the frequency from the average being approximately 2%). This constant frequency will be referred to as the normal frequency.

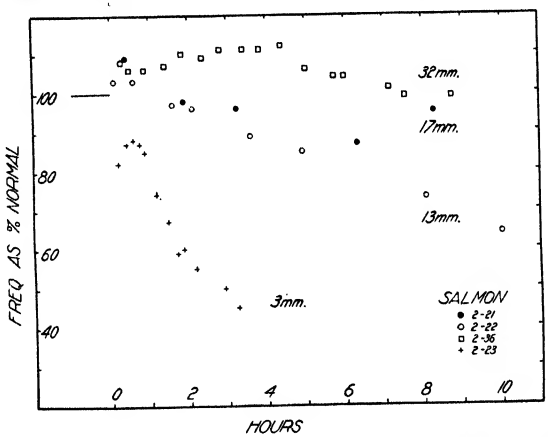


FIG. 2. *The effect of low partial pressures of oxygen on the frequency of the heart-beats in intact embryos of Salmo salar. At zero time the oxygen pressure was changed from approximately 150 mm. of mercury to the values indicated. The heart-beat frequency is expressed as a percentage of the normal frequency and each point is the average of observations made on four organisms at 6° C.*

Fig. 2 illustrates typical observations recorded upon subjecting salmon embryos to water containing oxygen at partial pressures below that in air. At 17 and 32 mm. the frequency did not depart consistently from the normal. At the other two oxygen concentrations shown, it fell with time. Apparently when supplied at partial pressures of 3 or 13 mm. oxygen cannot be utilized at the normal rate by the pacemaking cells, so that the frequency of the heart-beat falls. There must exist a pressure in the region of 13 to 17 mm. that is just sufficient to support the normal rate of oxygen consumption and

consequently the normal frequency. Such a pressure is usually designated the *critical* oxygen partial pressure. One may therefore speak of the lowest concentration of oxygen that will maintain the normal rate of the heart, as the critical partial pressure for heart-beat frequency.

As in the experiments described by Haywood *et al.* (4), the frequency fell more rapidly the lower the oxygen pressure, and the effects were completely reversible unless the oxygen lack was greatly prolonged. It will be noted that there is little, if any, indication that the frequency falls to an asymptotic level such as was seen when these preparations were exposed to oxidative poisons (1, 2, 3). Various degrees of auriculoventricular block developed as the frequency fell, and finally irregularities occurred even in the auricular beat. When this stage was reached the experiment was usually terminated (or the ability to recover was tested), as beyond this point the frequency observed probably does not represent accurately the activity of the pacemaking cells.

It is notable that the frequency does not fall instantaneously from its normal value to zero upon establishing conditions of oxygen lack, but that instead the change is gradual. It is quite inconceivable that it should require more than a few minutes to establish an equilibrium between the oxygen concentration to which the pacemaking cells are exposed, and one newly set up outside the organism. The comparatively slow rate of change of the frequency must, then, be a property of the pacemaking system after the partial pressure of oxygen has been brought to a new level. This inertia towards change suggests that the mechanism has, in effect, a "reserve of frequency". It is as if the heart rate is proportional to the concentration of a susbtance that is gradually utilized following the establishment of an oxygen concentration below that necessary for normal function. As would be expected, and as Fig. 3 indicates, this reserve is depleted more rapidly, the higher the temperature.

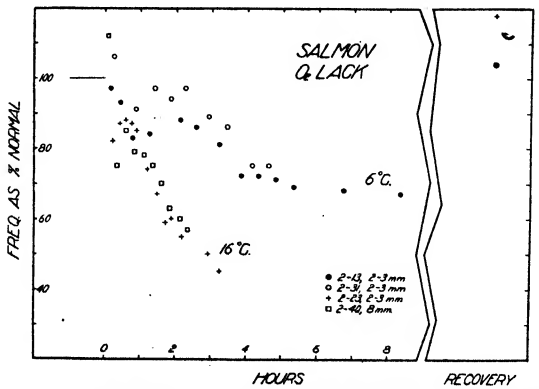


FIG. 3. *The effect of temperature on the rate at which the heart-beat frequency changes upon subjection of the intact embryo to low oxygen partial pressures.*

It is apparent from the data already given, that by subjecting groups of organisms to different partial pressures of oxygen, pressures can be distinguished that have no effect on the frequency, while others are clearly too low for the maintenance of the normal frequency. The results of a series of such observations are combined in Fig. 4. The ordinate of each point indicates

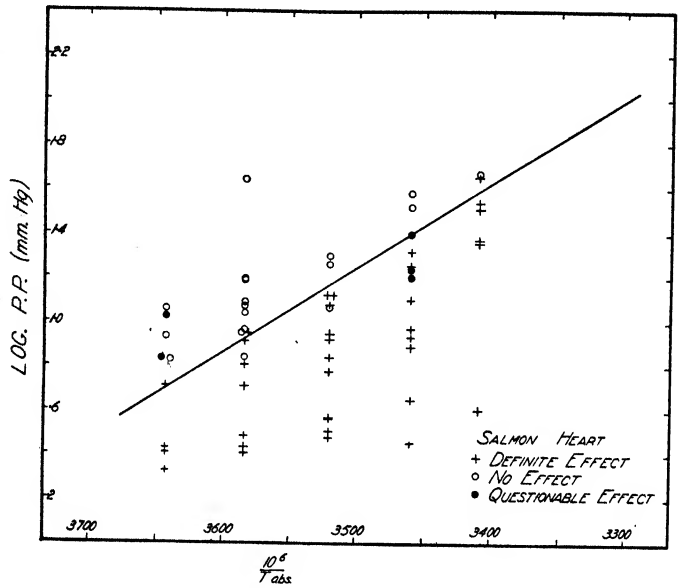


FIG. 4. *A composite plot of data obtained for the heart of the salmon embryo illustrating the critical oxygen partial pressure and its variation with temperature. See text for details.*

the logarithm of the partial pressure of oxygen throughout that experiment while the abscissa represents the reciprocal of the absolute temperature at which it was performed. Those experiments in which the average frequency of the heart-beats in the four embryos employed fell unmistakably as a result of the lowered oxygen concentration, are designated by crosses, those in which no effect was detectable, by circles. Borderline cases in which the effect was so slight as to be within the possible variation of a control are indicated by solid circles. The trend of the points is such that practically all of the crosses are below the line drawn through the data (in that region the oxygen concentration was below the critical value) while most of the circles lie above it; there, the experiments were preponderantly above the critical oxygen partial pressure. The disposition of the points is such that this line can be considered a fairly accurate representation of the variation of the critical partial pressure with temperature. The logarithm of the critical pressure can therefore be expressed as a linear function of the reciprocal of the absolute temperature. The data in Fig. 4 were obtained on embryos of Atlantic salmon. In Fig. 5 the equivalent data for embryos of speckled

trout are given. The critical pressures for these two species and the mode of its variation with temperature are grossly similar.

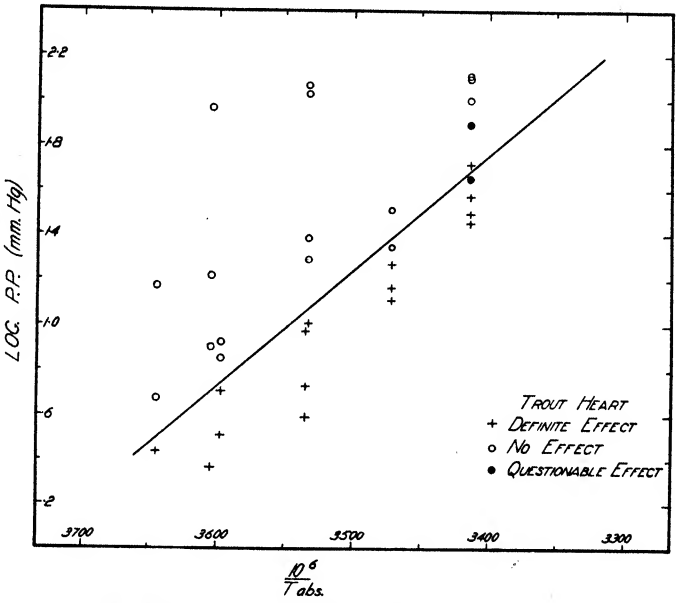


FIG. 5. *The data obtained for speckled trout treated as salmon (Fig. 4).*

The slopes of the lines in Figs. 4 and 5 are measures of the effect of temperature on the critical oxygen partial pressure. To evaluate the slopes the lines may be described by equations having the form

$$4.6 \log [O_2] = \frac{H}{T} + C \qquad (1)$$

where $[O_2]$ is the critical partial pressure of oxygen at the absolute temperature T, H is a constant evaluating the slope of the line and C is also a constant. H alone is significant in the present connection.

Some difference of opinion is possible with regard to the slope of the lines drawn in the two figures. For salmon, eight individuals who examined the data independently placed lines for which the value of H varied from 16,200 to 18,700. From the line shown $H=17{,}700$. Similarly, as judged by the same individuals, H for the data of Fig. 5 lies between 21,300 and 25,800, being 23,900 for the line drawn. It seems therefore that the estimation of the value of H from Figs. 4 and 5 may vary by $\pm 5\%$ (this being the average deviation of the value determined by each of the individuals from the average of the eight values).

It will be appreciated that the effect of temperature on the rate at which the frequency falls at low partial pressures of oxygen, complicates the decision as to whether or not a given pressure is above or below the critical value. If

the census to determine the effect of the low pressures is taken after a given duration of exposure, the critical pressure at low temperatures may appear to be relatively higher than the true value. This situation could result from the possibility that a partial pressure determined to be above the critical value at a low temperature, might have proved to be below it if a greater duration had been allowed. An artifact such as this will cause the critical values reported for the lower temperatures to be higher than the true values, and the effect of temperature will therefore appear less than it actually is. In the experiments on salmon, the average length of the exposure to reduced pressures was eight and one-quarter hours. The data given in Fig. 4 were obtained by examining the observations at the conclusion of each experiment. The results are not significantly different if considered after an exposure of only five and one-half hours. However, two hours after establishment of the lower pressure, the observations suggest a much smaller temperature effect. It is clear that the values recorded in the figure are free of any discrepancy that might be due to the slower changes of frequency at the low temperatures.

The data for trout are less complete, the average exposure to the low concentration of oxygen being only four and one-half hours. There is a definite possibility in this case that the indicated effect of temperature is smaller than the true value. H as determined for salmon and trout may be more different therefore than at present appears.

It will be suggested in the discussion that H is a quantitative characteristic of one step in the sequence of chemical reactions that constitute the pacemaking process (cf. 2). That step is the combination of oxygen with a respiratory catalyst, H measuring the effect of temperature on the equilibrium constant of the reaction.

The effect of temperature on the over-all velocity of the pacemaking system can likewise be estimated from the data obtained in this research, for the normal rate of the hearts is a measure of that velocity. It follows that from a consideration of the normal frequencies at the different temperatures, an indication is obtained of the effect of temperature on the velocity of the pacemaker. While H, and the equivalent constant ($=\mu$) descriptive of the temperature effect on the normal frequencies are probably not related in a simple fashion, it is of interest to compare them. In Fig. 6 the logarithms of the normal frequencies have been plotted against the reciprocal of the absolute temperature at which they were observed. Every point is the arithmetical average of the heart-beat frequencies in 16 embryos, each of which was observed at least four times over the control period of two hours or more. Since the normal frequencies were found to change by as much as 25% with the age of the organisms, only those data obtained at a given age can be compared strictly. The determinations indicated by crosses are comparable, for a given species, having been obtained over a period not exceeding 10 days. The points shown as circles are also comparable. All remaining observations were made over such a long interval that the change with age was operative and therefore these were not considered in fitting the

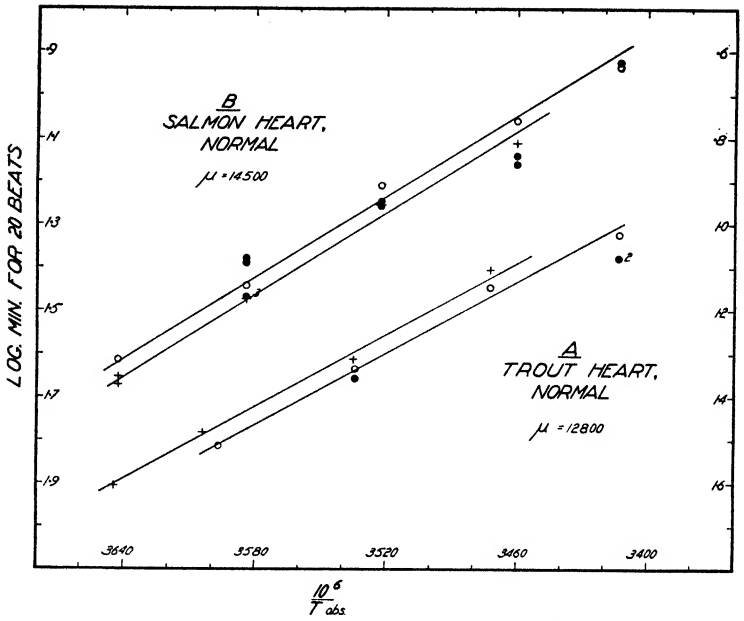


FIG. 6. *The effect of temperature on the normal frequency of the heart-beat in embryos of salmon and trout, respectively. The ordinate at the left of the figure refers to salmon, while that at the right refers to trout.*

straight lines. It is apparent that temperature does not affect the heart-beat frequency in exactly the same way in the two organisms. Moreover, the values of μ and of H for a given preparation may differ greatly, being, in trout, 12,800 and 23,900, respectively, and in salmon 14,500 and 17,400. Clearly, the circumstances that establish the effect of temperature on the critical partial pressure of oxygen are not identical with those that establish the magnitude of the temperature effect on the normal frequency. Ideally H may be pictured as a measure of the relation between temperature and the concentration of some component in the reaction chain through which oxygen is consumed. On the other hand μ indicates the effect of temperature on the velocity with which oxygen or its equivalent passes over that chain.

While the rise of the critical oxygen partial pressure with temperature may be a very general phenomenon (5), the critical pressure at a given temperature varies widely from one preparation to another (10). In this connection it is possible to compare the critical pressure for heart-beat frequency in trout and salmon embryos with that in *Fundulus*. For the former at 22° to 23° C. the critical pressure is approximately 60 mm. of mercury while, as judged from the paper by Fisher and Cameron (2), it cannot be greater than about 25 mm. in *Fundulus*.

Though the effect of age was not examined particularly in the present investigation, the data suggest that the critical partial pressure at a given

temperature may rise significantly as the organisms become older. In addition, the whole sequence of events upon exposure to oxygen lack occurs more rapidly as the organisms grow older. These changes with age can undoubtedly be considered as examples of the rather general fact that embryonic organisms are more resistant to asphyxial conditions than are adults.

Discussion

The precise significance to be attached to the magnitude of the temperature characteristic that is reported here, depends on the mechanism that is responsible for establishing a critical oxygen partial pressure in these preparations. The more obvious of the two conditions that could theoretically lead to the existence of a critical pressure is of course the restriction imposed on oxygen consumption by the physical process of diffusion. In order to be utilized at the centre of a mass of cells oxygen must diffuse through the mass from the source. For a given rate of utilization there may therefore be a limiting partial pressure, the critical pressure, which will just suffice to produce the necessary rate of diffusion. With the rate of utilization determining the critical pressure, the effect of temperature on a critical pressure will be determined to a great degree by the effect of temperature on the rate of utilization.

On the other hand, the existence of a critical oxygen partial pressure as a property of the chemical reactions into which oxygen enters in the cells, is implied (cf. 10) in the quantitative theory of cell respiration developed by Warburg (11). In all respects that have been examined (2) it has been shown that heart-beat frequency can be substituted for oxygen consumption in this theory. It is implied therefore, that the frequency of the heart-beat also should exhibit a critical oxygen pressure, whose significance is identical with that for the critical pressure observed for oxygen consumption. Moreover, the data of Stotz, Altschul, and Hogness (8) show that the isolated cytochrome oxidase system, which is believed to be of very general importance in the respiration of all aerobic cells, exhibits a critical pressure in ranges of oxygen concentration that are physiologically significant.

From the standpoint of cellular respiration the critical pressure is a measure of the affinity of an intracellular oxidative catalyst for oxygen, i.e., a measure of the equilibrium constant (K) of the mass law equation applying to the combination of oxygen with the catalyst. The effect of temperature on the critical pressure is thus a measure of the effect of temperature on K. This constant, and hence the critical pressure, should be related to temperature by an expression of the van't Hoff isochore (10). That formula is indistinguishable from Equation 1 which, as Figs. 4 and 5 show, can be employed to describe the relation between the critical pressure and the temperature. The data are therefore consistent with the view that the critical pressure is a chemical property of the cellular oxidative systems.

It has not been possible to obtain experimental proof that diffusion either does or does not take part in determining the critical partial pressures that have been observed. However, the organisms used possessed an intact circulatory system so that the actual diffusion distances were probably of the order of the diameter of a single cell. It seems much more likely therefore, that the critical pressures recorded are not due to diffusion but are a property of the chemical mechanism in the pacemaking cells of the heart.

The data described in this paper reveal the precise conditions that must obtain with regard to the partial pressure of oxygen in order that normal functioning of the heart in these embryos may proceed. With the observations in mind, the common practice of lowering the temperature when conditions occur that may be adverse in relation to the oxygen supply, is seen to be advantageous in two respects. It is well known of course that the quantity of oxygen consumed per unit time is smaller, the lower the temperature. It is to be recognized now that lowering the temperature also decreases the critical oxygen partial pressure. As a consequence a larger fraction of the quantity of oxygen present under given conditions can be utilized at low temperatures before effects of oxygen lack occur than can be utilized similarly at higher temperatures. This fact is made particularly striking by a comparison of the actual conditions at two temperatures. From Fig. 5 it can be seen that at 25° C. trout embryos, when placed in water initially saturated with air, could utilize only 30% of the oxygen present before the partial pressure of that gas would be so reduced as to interfere with the heart rate. At 5° C. on the other hand, some 97% of the oxygen could be used before the functioning of the heart would be affected by the low partial pressure.

Though obtained for a particular organ, the heart, these results can probably be taken as indicative of the situation in all organs and, in fact, as characteristic of the entire individual. It should be noted, however, that great distortion of the comparatively simple picture found here is at least theoretically conceivable. If, for example, the passage of oxygen from the external environment involves a respiratory pigment such as haemoglobin (though there was undoubtedly haemoglobin present in the circulatory system of the embryos, it seems unlikely that the pigment played a significant role in the stages used), then the properties of this pigment might result in the appearance of quite a different picture. For such reasons a wide generalization to intact organisms on the basis of the data presented is not possible.

Acknowledgment

Salmon and trout eggs were obtained from the Fish Culture Branch, Dominion Department of Fisheries, through the courtesy of Mr. J. A. Rodd. The author wishes also to express his indebtedness to Mr. Herbert Pearen for a supply of trout eggs, and his appreciation of the assistance given by Mr. Stephen Clare during the performance of the experiments.

References

1. ARMSTRONG, C. W. J. and FISHER, K. C. J. Cellular Comp. Physiol. 16(1) : 103-112. 1940.
2. FISHER, K. C. and CAMERON, J. A. J. Cellular Comp. Physiol. 11(3) : 433-454. 1938.
3. FISHER, K. C. and ÖHNELL, R. J. Cellular Comp. Physiol. 16(1) : 1-13. 1940.
4. HAYWOOD, C., STEVENS, T. O., TEWINKEL, H. M., and SCHOTT, M. J. Cellular Comp. Physiol. 5(4) : 509-518. 1935.
5. KEMPNER, W. Cold Spring Harbor Symposia Quant. Biol. 7 : 269-289. 1939.
6. LUND, E. J. Proc. Soc. Exptl. Biol. Med. 19 : 63-64. 1921.
7. ROEBUCK, J. R. Rev. Sci. Instruments, 3(2) : 93-100. 1932.
8. STOTZ, E., ALTSCHUL, A. M., and HOGNESS, T. R. J. Biol. Chem. 124(3) : 745-754. 1938.
9. SUTTON, F. A systematic handbook of volumetric analysis. 12th ed. P. Blakiston's Son and Company, Inc., Philadelphia. 1935.
10. TANG, P.-S. Quart. Rev. Biol. 8(3) : 260-274. 1933.
11. WARBURG, O. Biochem. Z. 189 : 354-380. 1927.

THE LIFE CYCLE OF *APANTELES CARPATUS* (SAY) (HYMENOPTERA: BRACONIDAE), A PARASITE OF THE WEBBING CLOTHES MOTH, *TINEOLA BISSELLIELLA* HUM.[1]

By A. Murray Fallis[2]

Abstract

This parasite has been reared from larvae of the webbing clothes moth obtained from different localities as well as experimentally. All parasites obtained by natural and experimental infections were females. Oviposition occurred and parasites developed in host larvae weighing 1.6 to 6.8 mg. The parasites oviposited more readily in a host enclosed in a case, especially if the case contained fecal pellets of the host. Eggs were deposited in various parts of the host. A single parasite developed to maturity even though several eggs may have been deposited in the host, each by a separate "thrust" of the ovipositor. Morphological features of the larvae are illustrated. The rate of development varied even at constant temperature. The average length of the life cycle at 27° C. was 26 days but at 20° C. it required several months. Experiments were carried out to determine the factors responsible for the variation in the rate of development. The parasite larva, upon emerging from the host, usually spins a white, silken cocoon, although metamorphosis was sometimes completed even though no cocoon was produced.

Apanteles carpatus (Say) was reared from the case-making clothes moth, *Tinea pellionella* L. in Connecticut by Mr. W. D. Kearfott in 1905 (Viereck *et al.* (3)). The present study reports observations on its habits and life history in larvae of the webbing clothes moth, *Tineola bisselliella* Hum. A culture of clothes moths being maintained at the Ontario Research Foundation was almost completely decimated by this parasite. Moth larvae received from three other localities in Toronto were also found to be naturally infected by it.

Mr. Muesebeck of the Bureau of Entomology and Plant Quarantine, Washington, D.C., who kindly identified the parasite, states (personal communication) that it has a world-wide distribution. Viereck *et al.* (3) reported that it also has been bred from the white-marked tussock moth, *Hemerocampa leucostigma* Smith and Abbot, but Muesebeck (personal communication) states, "This is almost certainly incorrect. The species is not infrequently reared from clusters of tussock moth cocoons, not as a parasite of that lepidopteran but of one of the several tineid scavengers that occur in such situations."

Habits of Adults

Adult parasites were kept at approximately 20° C. and fed on diluted honey. Each was placed in a separate vial in the bottom of which was absorbent cotton, partly moistened with honey. A large number were kept alive in vials for two to three weeks, although some died much sooner. Many of the parasites drank thirstily from drops of water when they were made available to them.

[1] *Manuscript received July 18, 1941.*

Contribution from the Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Ont.

[2] *Research Fellow.*

Adults (Fig. 1) are attracted to light of certain intensities, especially just after emergence from the cocoon, for they invariably flew to the window when they escaped. The antennae are held straight in front when the insect is resting but they are curved ventrally when it moves about. The ovipositor can be seen protruding from the posterior end of the abdomen. The characteristic fore wing is illustrated in Fig. 2.

These insects are excitable and easily disturbed. At times they showed no inclination to oviposit, although a short time later they might attempt to do so. The ovipositor is illustrated in Fig. 3. This photograph shows a lateral view of the abdomen of an insect which was cleared and mounted in balsam. The stylets of the ovipositor as well as the sheaths are visible. The stylets are held within the sheaths when the insect is at rest but are sharply bent ventrally during oviposition so that the extremities of the sheaths move toward the base of the stylets and appear to act as a guide for them.

Oviposition

Eggs are produced parthenogenetically. The sense of odour or touch, or both, appeared more effective in directing the parasite to a host than the sense of sight, for the host frequents dark places yet, as previously mentioned, the parasite is attracted to light. Moreover, moth larvae were attacked more readily if encased, especially if fecal pellets had been used in the construction of the cases. "Thrusts" were made even into empty cases and frequently at a single fecal pellet. Only a few seconds were required for oviposition, which took place in any part of the host. A parasite would deposit several eggs in the same host, a separate "thrust" of the ovipositor being required to deposit each egg, although sometimes no egg was left. The "thrusts" sometimes caused the larva within a case to emerge. All the eggs usually hatched but of the resulting larvae only one grew to maturity.

The maximum lengths of living larvae found in the same host were 0.5 mm. and 0.3 mm., respectively, but in most cases by the time one of the larvae had

---

EXPLANATION OF FIGURES

PLATE I

All figures represent stages in the life cycle of Apanteles carpatus (Say).

FIG. 1. *Adult. ca. 5×.*
FIG. 2. *Fore wing of adult. ca. 16×.*
FIG. 3. *Lateral view of abdomen to show ovipositor. ca. 16×.*
FIG. 4. *Appearance of egg when deposited in host. ca. 190×.*
FIGS. 5, 6, 7. *Successive stages in development of eggs. ca. 190×.*
FIG. 8. *Ventral view of larva shortly after emerging from egg. ca. 190×.*
FIG. 9. *Lateroventral view of larva 0.5 mm. long. ca. 110×.*
FIG. 10. *Lateral view of larva 0.5 mm. long. ca. 110×.*
FIG. 11. *Laterodorsal view of larva 0.6 mm. long. ca. 110×.*
FIG. 12. *Lateral view of larva 1.5 mm. long. ca. 60×.*
FIG. 13. *Silken cocoon from which parasite has emerged. Note cap removed from one end. ca. 4×.*

1

4

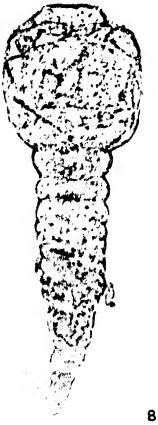
5

2

6

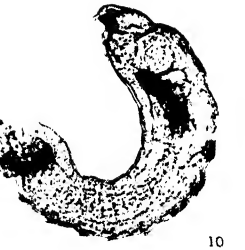
3

7



8

9



10

13

12

11

reached a length of 0.5 mm., the others were all dead. Eggs may be laid by a parasite the same day that it has emerged from a cocoon.

Development and Morphology of Larvae

The size of the eggs (Fig. 4) immediately following oviposition, based on a measurement of 10 eggs, is: average length—0.28 mm., maximum—0.30 mm., minimum—0.24 mm.; average width of expanded portion—0.035 mm., maximum—0.044 mm., minimum—0.024 mm. Development of the egg is rapid (Figs. 5, 6, 7) and a larva (Fig. 8) hatches in a few days, the actual rate depending on the temperature.

Internal structure in the first stage larva is not very apparent. The broad cephalothoracic region compared to the narrower abdominal region is noticeable. A strong pair of mandibles is attached to the ventral surface just ventral to the oral aperture. The so-called antennae (Fig. 14), which are anterior and dorsal to the mandibles, are small, movable, teat-like structures, with a small spine near the base of the dorsal surface of each. An irregular number of spines are arranged in rows on the dorsal surfaces of the eight (occasionally, nine) posterior abdominal segments (Fig. 14). A long caudal appendage is also apparent.

Larvae, 0.5 to 0.6 mm. in length, are shown in Figs. 9, 10, 11. The mandibles and antennae are both distinguishable in Fig. 9, but the shape and relative position of the antennae are shown to better advantage in Fig. 10. A prominent caudal vesicle is noticeable. The caudal appendage appears relatively smaller than in the earlier stage. The position of the brain and gonads may also be seen in Fig. 10, although they are better illustrated in Fig. 11. These various structures will be located more readily by reference to Figs. 14 and 15.

The brain, nerve cord, intestine, heart, and ovaries are well developed in larvae 1.5 mm. long or even smaller (Figs. 12 and 15). The heart and nerve cord were observed in some specimens 0.7 to 0.8 mm. long. The silk glands and ducts (Fig. 15) are also formed, although the former become more extensive later. Larvae measured 3 to 4 mm. in length upon emergence from the hosts (Fig. 17) and in those 3 mm. long (Fig. 16) the main branches of the tracheal system are visible. The mouth parts are more difficult to distinguish than in smaller forms. The mandibles are relatively smaller and, on the posteroventral border of each, there is a row of small teeth. The silk glands are extensive. The anlages of some of the appendages are visible in stained specimens. Minute spines occur over the entire body.

Rate of Development

The rate of development of the parasite varies even at a constant temperature. The minimum, maximum, and average time, at 27° C., between the deposition of eggs in 81 moth larvae and the emergence of mature parasite larvae from these hosts was 13, 45, and 20 days, respectively. The minimum

time at 24° C. was 20 days, the maximum, 154. The average for this temperature is not given, as only six parasites were reared and, although five of them emerged from their hosts in approximately 30 days, the sixth took much longer. Development at room temperature requires several months.

The growth of some larvae at 27° C. was as rapid as that shown in Table I. This is probably an extreme rate, the average being somewhat slower.

TABLE I

GROWTH OF LARVAE

Time, days	Size of larvae, mm.	Time, days	Size of larvae, mm.
1	Egg developing	5	0.48
2	Egg developing	6	0.72
3	0.36	7	1.52
4	0.40	10	4.0

The average time spent in the pupa stage (including the prepupa of some authors) at 27° C was eight days for 40 individuals, at 24° C., 11 days for five individuals, and at 20° C. (approximately), 17 days for three. Therefore, at 27° C. the life history should be completed in an average of 28 days. However, only 44 of the parasites completed their metamorphosis successfully following pupation, and the average length of their complete cycle at 27° C. was 26 days. This means that more of those parasites completed their cycle when the larval period was short than when it was more than the average length.

The parasite, after emerging from its host, spins a white, silken cocoon (Fig. 13) within the case in which the host is lying. Any attempt to spin a cocoon when the host is not within a case is unsuccessful, apparently because of the absence of a framework about it to assist in the process. It is suggested by these studies that a larger percentage of the parasites that pupate within host cocoons succeed in metamorphosing to adults than do those that are not in cocoons. Over 50% of the former changed to adults whereas only one out of six of the latter did so. The imago escapes from the cocoon by cutting a symmetrical opening at one end and then pushing the cap thus formed (Fig. 13) out of its way.

EXPLANATION OF FIGURES

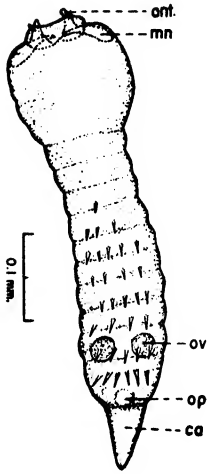
FIG. 14. *Outline drawing of first larval stage, ventral view.*

FIG. 15. *Drawing, somewhat diagrammatic, of 1.5 mm. larva, lateral view.*

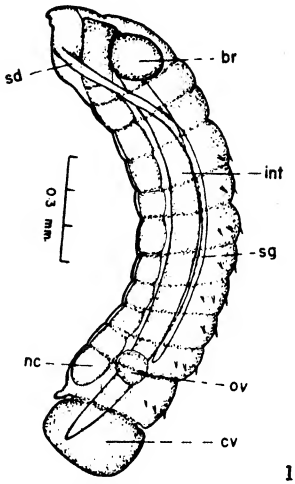
FIG. 16. *Main branches of tracheal system of 3 mm. larva as seen in lateral view.*

FIG. 17. *Drawing, somewhat diagrammatic, of 3 mm. larva, ventral view.*

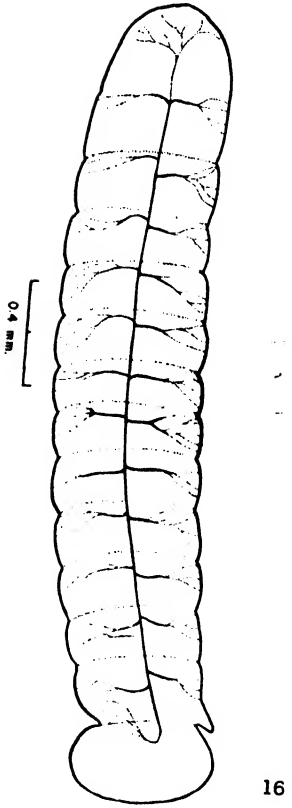
Abbreviations: anl., anlage of appendages; ant., antenna; br., brain; ca., caudal appendage; cv., caudal vesicle; com., circumoesophageal commissure; int., intestine; md., mn., mandible; nc., nerve cord; op., opening for caudal vesicle; or., oral aperture; ov., gonad; sd., silk duct; sg., silk gland.


ant.
mn
0.1 mm.
ov
op
ca

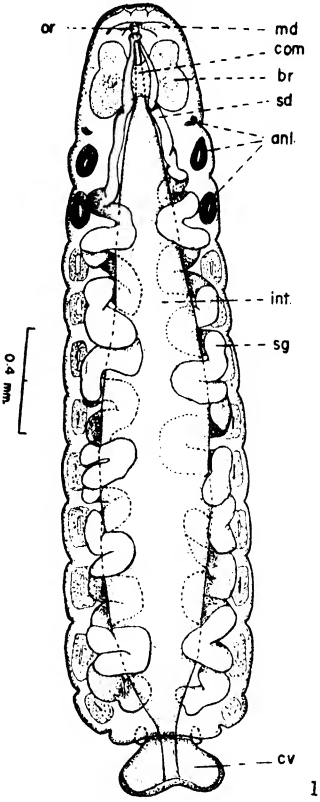
14


sd
br
int
0.3 mm.
sg
nc
ov
cv

15


0.4 mm.

16


or
md
com
br
sd
ant.
int.
sg
0.4 mm.
cv

17

Parasites were reared from hosts weighing 1.6 to 6.8 mg. There was no indication from experimental infections that development was faster in the larger hosts. There was no appreciable difference in the rate of development of parasites in hosts that had received several "thrusts" of the ovipositor as compared to those that received a single "thrust". Parasitic larvae emerged in an average of 19 days from 22 hosts in the former group and in an average of 18 days from 34 hosts in the latter group. It is worth noting that Moss (2) found that *Apanteles glomeratus* L. developed as rapidly in a host containing 100 parasites as in one that contained only 20.

The average time from deposition of the eggs until the emergence of parasites from 15 hosts receiving one "thrust" of the ovipositor in the anterior region of the body was 21 days, whereas it was only 17 days in the case of nine hosts that received one "thrust" in the posterior part of the body. This difference may not be significant as the eggs were not all deposited by the same parasite and it will be seen from Table II that the average rate of development of the progeny from one parent may differ from that of another.

TABLE II

AVERAGE RATE OF DEVELOPMENT OF PROGENY OF FIVE PARASITES

—	Parasite No.				
	1	2	3	4	5
Number of hosts from which parasites emerged	8	9	7	8	12
Time (days) until emergence of parasite larvae					
Minimum	13	14	14	13	14
Maximum	53	31	19	30	22
Average	24	18	16	24	17

The table includes only those hosts from which larvae emerged. In many cases the host died before the parasites had completed their development. The hosts used in the experiments received "thrusts" by the parasites in different parts of their bodies. The results of this small experiment suggest that the average rate of development of a parasite may be partially dependent on its parentage.

Environmental humidity as well as temperature may affect development of the parasites (Table III).

TABLE III

DEVELOPMENT AT 27° C. AND THREE DIFFERENT RELATIVE HUMIDITIES

—	Relative humidity, %		
	30	45	75
Number of hosts infected	22	28	15
Percentage of hosts from which larvae emerged	77	57	60
Percentage of hosts from which adult parasites were obtained	59	28	26
Percentage of emerging larvae metamorphosing to adults	76	50	44

A relative humidity of 30% would appear, therefore, to be the more suitable for the development of the larvae as well as for metamorphosis into the adult parasite. The humidity might be expected to have the most influence on the pupal stage but the effect on larval development appears from the above to be equally marked. The effect of humidity on the parasite thus seemed to differ from that on the host itself, for Griswold and Crowell (1) found a relative humidity of 75% most favourable for the development of the webbing clothes moth larvae.

Acknowledgments

The author would like to acknowledge gratefully the kind assistance and co-operation of those who have made this work possible—Dr. H. B. Speakman, Director of the Foundation, for his advice and encouragement; Dr. S. Hadwen under whom the work was carried out; Dr. E. M. Walker for assistance in interpreting the morphology; Mr. C. F. W. Muesebeck for identifying the parasite, making helpful suggestions and reading the manuscript; Mr. Norman Law for assisting with many of the experiments and photographs.

References

1. Griswold, G. H., and Crowell, M. F. Ecology, 17 (2) : 241-250. 1936.
2. Moss, J. E. J. Animal Ecol. 2 (2) : 210-231. 1933.
3. Viereck, H. L., MacGillvray, A. D., Brues, C. T., Wheeler, W. M., and Rohwer, S. A. Connecticut Geol. Nat. Hist. Survey 5, Bull. 22 : 1-824. 1916.

ON THE USE OF THE pH VALUE AS A MEASURE OF THE FRESHNESS OF FISH MUSCLE TISSUE[1]

By F. Charnley[2] and D. H. Goard[3]

Abstract

The pH value of the aqueous liquors derived from fish muscle tissue is connected through statistical relations with the buffer action of the liquor, the log bacterial count of the sample, and with a subjective estimate of freshness determined on the basis of odour. In the case of pH and odour rating the relation is not a correlation but, instead, a linear relation between the means of a series of populations. By means of the latter it is possible from observations of the pH of the aqueous liquor in the sample to determine objectively the freshness of a parcel of canned chum salmon to any desired degree of accuracy by increasing the size, n, of the sample taken for examination.

The pH value possesses a number of advantages over other tests that have been proposed from time to time for measuring spoilage in fish or animal tissue. One of these is that the pH can be measured accurately; secondly, the test can be carried out rapidly; and thirdly, the pH, owing to the fact that it is apparently a function of the degradation or fission of all protein constituents in the tissue, is the most generally applicable of any of the chemical tests so far suggested for this purpose. As in other tests for spoilage, however, the pH of fish muscle tissue, or aqueous liquors derived therefrom, shows considerable variation from sample to sample, so that before the test can be utilized for the accurate measurement of spoilage, it is necessary to know not only the relation between the pH value and a criterion of freshness, but also the standard deviation of the pH in samples drawn from the same quality as regards freshness.

The two fundamental criteria available for testing the validity of the pH as a measure of freshness consist of (1) a chemical test based on the observation of Van Slyke, that, as primary changes proceed, the buffer capacity of the tissue decreases, and (2) bacteriological tests, in which the pH value is compared with bacterial counts as deterioration proceeds. In addition to the two fundamental criteria, however, there is a third and, practically, more important criterion than either of these for testing the validity of the pH as a measure of freshness, namely, the ratings of an experienced examiner based on the results of organoleptic tests, that is, on odour and other evidences of incipient decomposition. The investigation reported in this paper was concerned primarily with the relation between pH and this third criterion.

[1] *Manuscript received September 3, 1941.*

Contribution from the Canned Salmon Inspection Laboratory, Department of Fisheries, Vancouver, B.C.

[2] *Chief Chemist.*

[3] *Senior Laboratory Assistant.*

Relations between pH, Buffer Action, and Log Bacterial Count

The nature of the relations between the pH value of an aqueous suspension of the sample and the first two criteria of freshness can be readily illustrated by the results of Stansby and Griffiths (6). In their study of the deterioration in quality of freshly caught haddock these authors made bacterial counts on the samples and measured the buffer capacity of a suspension of 5 gm. of the muscle tissue in 100 cc. of water. The number of cubic centimetres of 0.0165 *N* hydrochloric acid required to bring the pH of the suspension to 6 was taken as a measure of the secondary changes in the fish tissue and was termed the *B* value, while the additional number of cubic centimetres of the acid required to bring the pH to about 4 was taken as a measure of the primary

TABLE I

RELATION BETWEEN pH AND $100/A$

pH	$100/A$														f
	3.06 to 3.55	3.56 to 4.05	4.06 to 4.55	4.56 to 5.05	5.06 to 5.55	5.56 to 6.05	6.06 to 6.55	6.56 to 7.05	7.06 to 7.55	7.56 to 8.05	8.06 to 8.55	8.56 to 9.05	9.06 to 9.55	9.56 to 10.05	
7.41 to 7.47														1	1
7.34 to 7.40						2		1							3
7.27 to 7.33															–
7.20 to 7.26					1	1									2
7.13 to 7.19						1	1		1						3
7.06 to 7.12				1	4			1	1						7
6.99 to 7.05			2	1	1	1	1	1							7
6.92 to 6.98			1	2	1	1	2								7
6.85 to 6.91	1		2	2		2	1				1				9
6.78 to 6.84			4	3											7
6.71 to 6.77		3	3	1											7
6.64 to 6.70		1	2	2	1										6
6.57 to 6.63			3												3
6.50 to 6.56			1												1
f	1	4	18	12	8	8	5	3	2	–	1	–	–	1	63

NOTE: $M_1 = 5.389$; $M_2 = 6.921$; $S_X = 1.156$; $S_Y = 0.2063$; $R = 0.6363$.

changes that had taken place in the sample. This was called the A value and was based on the results of Van Slyke (7).

Evidently, only the A value can properly be regarded as a measure of changes in the constituents of the muscle tissue, since the B value refers to different pH ranges and will tend merely to follow the titration curve. The two quantities most suitable for revealing the character of the relation between the pH value and freshness are therefore the A value and the log bacterial count. Since, however, the A value decreases with increasing pH value, it was found more convenient to employ the quantity $100/A$, that is, a measure of the buffer action (1) instead of the buffer capacity of the suspension.

Tables I, II, and III show the relations between the three quantities,

TABLE II

RELATION BETWEEN pH AND LOG BACTERIAL COUNT

pH	Log bacterial count											f
	2.8 to 3.2	3.3 to 3.7	3.8 to 4.2	4.3 to 4.7	4.8 to 5.2	5.3 to 5.7	5.8 to 6.2	6.3 to 6.7	6.8 to 7.2	7.3 to 7.7	7.8 to 8.2	
7.41 to 7.47								1				1
7.34 to 7.40									2			2
7.27 to 7.33												–
7.20 to 7.26								1			1	2
7.13 to 7.19					1				1			2
7.06 to 7.12						2	1	2				5
6.99 to 7.05						1		2	1			4
6.92 to 6.98				1	1	2	1	1	1			7
6.85 to 6.91	1		1	1		4	1			1		9
6.78 to 6.84	1		3	2			1					7
6.71 to 6.77	1	1	1		2	1			1			7
6.64 to 6.70					1	5	1					7
6.57 to 6.63			1			1	1					3
6.50 to 6.56						1						1
f	3	1	6	4	5	17	6	7	6	1	1	57

NOTE: $M_1 = 5.474$; $M_2 = 6.894$; $S_X = 1.137$; $S_Y = 0.2033$; $R = 0.4796$.

pH, log bacterial count, and $100/A$ derived from the data of Stansby and Griffiths. From an inspection of these data, it is apparent that there are definite relations between these three variates. As measured by the values of the correlation coefficient, the relations involving bacterial count are not as close as might have been expected, but that connecting pH and $100/A$ shows a relatively close correspondence between the two variates.

In the data of Tables I, II, and III the usual significance cannot be attached to the values of R, since there is no evidence that the samples have been drawn from the same quality as regards freshness. In fact, by virtue of the differences in treatment of the various sets of samples, it may be safely inferred that the samples represent varying degrees of quality in this respect. Hence, the data are very probably composite distributions and the values of the correlation coefficient only apparent correlations. The data, unfortunately, are not sufficiently numerous to allow a satisfactory investigation of the true character of the relations between the three variates, but in the

TABLE III

RELATION BETWEEN $100/A$ AND LOG BACTERIAL COUNT

$100/A$	Log bacterial count											f
	2.8 to 3.2	3.3 to 3.7	3.8 to 4.2	4.3 to 4.7	4.8 to 5.2	5.3 to 5.7	5.8 to 6.2	6.3 to 6.7	6.8 to 7.2	7.3 to 7.7	7.8 to 8.2	
8.06 to 8.55						1						1
7.56 to 8.05												–
7.06 to 7.55								1	1			2
6.56 to 7.05								2	1			3
6.06 to 6.55					1	1	1	1	1			5
5.56 to 6.05				1		2		2	2	1		8
5.06 to 5.55					1	4	1		1	1	1	9
4.56 to 5.05	1		1	1		4	3	1	2			13
4.06 to 4.55	1		5	2	1	7	1					17
3.56 to 4.05		1			2	1						4
3.06 to 3.55	1											1
f	3	1	6	4	5	20	6	7	8	2	1	63

NOTE: $M_1 = 5.556$; $M_2 = 5.125$; $S_X = 1.141$; $S_Y = 0.9926$; $R = 0.5270$.

case of the variates pH and $100/A$, the relation appears to be very similar to that connecting the pH value and examiner's rating discussed below.

Relation between pH and Examiner's Rating

The results employed in determining the relation between the pH value and examiner's rating for freshness were derived from samples of canned chum salmon (*Oncorhynchus Keta* Walbaum, 1792) packed late in the season between September 15 and October 31, 1936. The pH values are the readings given by the aqueous liquid in the samples and were determined by means of a Beckman glass electrode apparatus. The examiner's ratings were, as far as possible, based on the odour of the interior of the sample. These were determined immediately before the pH value of the aqueous liquid in the sample was taken and were recorded as follows: A = good, B = better than average, C = average, D = stale, and E = tainted.

Table IV shows the distributions of the pH of the free aqueous liquid in the various samples tabulated according to packing date. On assigning the

TABLE IV

DISTRIBUTION OF pH OF FREE AQUEOUS LIQUID IN SAMPLES OF CANNED CHUM SALMON PACKED BETWEEN SEPT. 15 AND OCT. 31, 1936, IN THE FRASER RIVER AND VANCOUVER ISLAND AREAS

pH	Packing date																	Total
	September					October												
	15	18	19	24	25	11	14	15	16	17	22	23	24	27	28	30	31	
6.61 to 6.63												1						1
6.58 to 6.60																		
6.55 to 6.57												1						1
6.52 to 6.54												1	1					2
6.49 to 6.51																		
6.46 to 6.48											1	1		2	1			5
6.43 to 6.45	2								1	1	2	3			2			11
6.40 to 6.42		1					1				2	4	3	1				12
6.37 to 6.39					3		2	2	1	1	3	1	2				1	16
6.34 to 6.36					1		2	1	4	4	4		1					17
6.31 to 6.33			1		3		1	2	4	6	2		1					20
6.28 to 6.30					2	2	2	2	7	5	1	1	1	1				24
6.25 to 6.27		1			5	1			4	3	1		2			1		18
6.22 to 6.24				2	3		1	2		2								10
6.19 to 6.21				1	1					2								4
Total	2	2	1	3	18	3	9	9	21	24	16	13	11	4	3	1	1	141

numbers $A = 1$, $B = 2$, etc., to the examiner's ratings, the corresponding distributions of examiner's ratings shown in Table V are obtained. From an inspection of the data in these two tables, it will be observed that in both instances there is a suggestion of a seasonal trend. As they stand, however, the data are almost useless for practical grading purposes since, in the first place, the trends are incomplete, secondly, the trends, if any, cannot be

TABLE V

DISTRIBUTION OF ODOUR RATING IN SAMPLES OF CANNED CHUM SALMON PACKED BETWEEN SEPT. 15 and OCT. 31, 1936, IN THE FRASER RIVER AND VANCOUVER ISLAND AREAS

Odour rating	Packing date																	Total
	September					October												
	15	18	19	24	25	11	14	15	16	17	22	23	24	27	28	30	31	
5												8		2				10
4	2	2	1				1		6	7		2	4	2	3	1	1	32
3					2	3	7	7	11	9	13	3	7					62
2				3	16		1	2	4	8	3							37
Total	2	2	1	3	18	3	9	9	21	24	16	13	11	4	3	1	1	141

adequately described by means of simple regression functions, and, thirdly, even if the regression functions were known, there is no evidence to show that such trends will be exactly repeated in future, or that samples packed in other districts by other canneries will be exactly described by these trends. To obtain data that will be suitable for practical grading purposes, therefore, the relation connecting the variates pH and odour rating will be required.

TABLE VI

RELATION BETWEEN EXAMINER'S RATING (ODOUR) AND pH OF AQUEOUS LIQUID IN SAMPLES OF CANNED CHUM SALMON

pH (Y)	Odour (X)				f
	B 2	C 3	D 4	E 5	
6.60 to 6.649				1	1
6.55 to 6.599				1	1
6.50 to 6.549			1	1	2
6.45 to 6.499		2	7	2	11
6.40 to 6.449		8	6	3	17
6.35 to 6.399	8	15	3	1	27
6.30 to 6.349	14	17	7	1	39
6.25 to 6.299	7	15	7		29
6.20 to 6.249	7	5	1		13
6.15 to 6.199	1				1
f	37	62	32	10	141

NOTE: $M_1 = 3.1064$; $M_2 = 6.3445$; $S_X = 0.8728$; $S_Y = 0.07940$; $R = 0.4971$.

Table VI shows values of pH tabulated according to odour rating. Comparison of the data listed in this table with the example of a linear composite bivariate normal distribution given by Charnley (2) suggests that the distribution relating pH and odour rating is also of the latter type.

If this hypothesis is correct, then from the data given in Table VI it is immediately evident that the slope m of the line relating the means of the component distributions is greater than zero. Consequently, if the variance in pH estimated from the individual codes, that is, from samples packed by the individual canneries on different days, is compared with the variance in pH given by the composite distribution (Table VI), there should be a significant difference in the two values.

The details of the subgroups from which the variance in pH in the individual populations was estimated for the purpose of this comparison are shown in Table VII. As will be seen from the table, the samples were packed at

TABLE VII

SUMS OF SQUARES OF pH AND ODOUR RATING IN INDIVIDUAL SUBGROUPS

Company	Cannery	Packing date	Sample size	nS_Y^2	ΣXY	nS_X^2
A	I	Sept. 15	2	0.00005	0.....	0.....
	I	Sept. 18, 19	3	0.01447	0.....	0.....
	I	Sept. 24	3	0.00127	0.....	0.....
	I	Sept. 25	3	0.00027	0.....	0.....
	I	Oct. 11	3	0.00127	0.....	0.....
	I	Oct. 14	9	0.02996	−0.02000	2.0000
	I	Oct. 15	9	0.02762	−0.00672	1.5556
	I	Oct. 16	9	0.02960	0.06452	2.2224
	I	Oct. 17	12	0.01609	0.06322	2.6668
	I	Oct. 22	6	0.01393	0.02180	1.3334
	I	Oct. 23	3	0.00127	0.....	0.....
	I	Oct. 24	3	0.00006	0.....	0.....
	I	Oct. 27	2	0.02000	0.....	0.....
	I	Oct. 28	3	0.00080	0.....	0.....
	I	Oct. 30, 31	2	0.00500	0.....	0.....
Total			72	0.16166	0.12282	9.7782
B	II	Sept. 25	15	0.03198	−0.03813	1.7335
	II	Oct. 16	12	0.00769	0.01100	3.0000
	II	Oct. 17	12	0.05520	−0.01960	2.9168
	III	Oct. 22	10	0.02285	−0.00300	0.9000
	III	Oct. 23	10	0.08521	0.13480	1.6000
	III	Oct. 24	8	0.02640	0.....	2.0000
	III	Oct. 27	2	0.00245	0.....	0.....
Total			69	0.23178	0.08507	12.1503
A + *B*			141	0.39344	0.20789	21.9285

three different canneries, two of the latter being operated by the same company. On applying Pearson's formula (4), namely,

$$\sigma_e^2 = \frac{1}{N-k} \sum_{t=1}^{k} \sum_{i=1}^{n_t} (x_{it} - \bar{x}_t)^2 ,$$

to the sum of the squares of the second variate (pH) given in Table VII it is found that $\sigma_y^2 = 0.003306$ pH units^2. Hence, if for convenience the scale of the second variate is so changed that 0.05 pH unit = 1 unit in the new scale, $\sigma_y^2 = 1.3225$.

The composite character of the distribution given in Table VI is now easily demonstrated by applying Sheppard's correction to the value of S_Y^2 calculated from the combined data and comparing (3) the two variances S_Y^2, σ_y^2, as shown in Table VIII. The result $P = 0.0005$ shows very definitely that the distribution of pH and odour rating given in Table VI is a composite distribution.

TABLE VIII

COMPARISON OF STANDARD DEVIATIONS IN pH ESTIMATED FROM INDIVIDUAL SUBGROUPS AND FROM COMBINED DATA

Distribution	D.f. (n)	Mean square	Log$_e$ S.D.	$1/n$
Single	119	1.3225	0.1398	0.008403
Composite	140	2.4383	0.4457	0.007143
			−0.3059	0.015546

NOTE: $z = -0.3059$; $\sigma_z = 0.08816$; *normal var.* = 3.4698; $P = 0.00052$.

Owing to the very broad categories in which the examiner's ratings occur, the component populations in the composite distribution given in Table VI differ considerably from normal with respect to the independent variate (examiner's ratings). Hence, we cannot legitimately estimate r by Fisher's (3) method, that is, by weighting corresponding values of z in the individual subgroups inversely as their variances. Also, for the same reason, the equation (2),

$$\mu_4' - m^4\mu_4 = 3(\mu_2')^2 - 3m^4(\mu_2)^2$$

cannot be safely applied to these data for calculating the value of m, the slope of the line of relation. The formulae given by Charnley (2), however, for calculating m and S_{YL}^2, the vertical variance around the line of relation, are rigidly applicable to all types of linear, composite, bivariate distributions, so that we may estimate the value of m and test the hypothesis regarding the linearity of the data of Table VI independently of any assumptions concerning the functional form of the data.

The equation giving the value of m is

$$\frac{S_Y^2 - \sigma_y^2}{S_X^2 - \sigma_z^2} = m^2 ,$$

in which S_X^2, S_Y^2, σ_z^2, and σ_y^2 are, respectively, the variances of the composite and component distributions. Applying Pearson's formula to the sum of the squares for odour rating given in Table VII we obtain as an estimate of the variance of the odour rating in the individual subgroups, $\sigma_z^2 = 0.1843$. The numerical values required in calculating m from the above equation are

accordingly, $S_X^2 = 0.7617$, $S_Y^2 = 2.4383$, $\sigma_x^2 = 0.1843$, and $\sigma_y^2 = 1.3225$. The substitution of these values in the equation gives $m^2 = 1.9325$ so that $m = 1.3901$. Expressed in the new units the line of relation is therefore a line passing through the point (0.1064, 0.3901) and having the slope $m = 1.3901$. The position of this line relative to the distribution given in Table VI is shown graphically in Fig. 1.

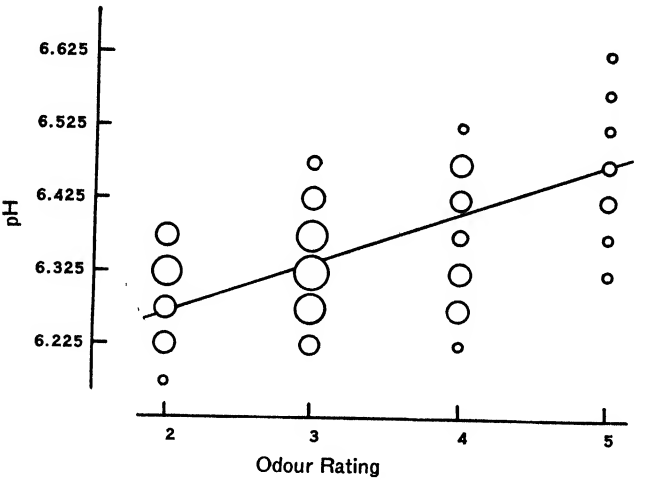


FIG. 1. *Diagram showing line of relation calculated from the data of Table VI. Areas of circles are approximately proportional to corresponding frequencies of single measures.*

Test of Linearity of Relation Between pH and Odour Rating

In view of the broad categories in odour rating and the consequent lack of normality of this variate in the component populations, the safest procedure in testing the hypothesis as regards linearity is to test averages of samples drawn from the individual subgroups. Averages, even of low sample sizes, such as two or three, will be more nearly normal than the single measures and in this case will probably be sufficiently close to normal to give a reasonably reliable test of the hypothesis, whether we apply Fisher's method of analysis of variance to the variance around the line of relation or set sampling limits on the averages.

The latter method was chosen for testing the averages of samples drawn from the individual subgroups listed in Table VII, since by applying Tchebycheff's inequality (5) this method can be made independent of the nature of the functional forms of the distributions of single measures or averages around the line of relation. To obtain reliable results by this method, however, it is necessary to know that the individual subgroups are reasonably homogeneous as regards the variances of the two variates and the correlation between the variates.

To ensure that this last condition was approximately met, the data of Table VI were therefore segregated according to companies. From Table IX it will be seen that the two groups of data are definitely non-homogeneous as regards variance in pH among single samples. Accordingly, to test the linearity of the relation between pH and odour rating, sampling limits were applied separately to the two sets of averages derived from the individual companies.

TABLE IX

TEST OF HOMOGENEITY OF VARIANCE IN pH IN SAMPLES PACKED BY COMPANIES *A* AND *B*

Company	D.f.	Sum of squares	Mean square	Log$_e$ S.D.	$\frac{1}{n}$
A	57	0.1617	0.002837	−0.6299	0.01754
B	62	0.3934	0.006345	−0.2274	0.01613
				−0.4025	0.03367

NOTE: $z = -0.4025$; $\sigma_z = 0.1297$; *normal var.* $= -3.1033$.

Table X shows the values of averages of samples of three drawn from the subgroups packed by the first of the two companies, that is, at one of the three canneries from which the samples were derived, while Table XI shows the corresponding data derived from samples packed by the two canneries of the second company. From the first of these tables we find that the value of $\Sigma(y - mx)^2$ is 0.05331, and from the second, 0.08221. From Pearson's formula the corresponding values of S_{YL} are therefore 0.04922 and 0.06257, respectively. These, it will be observed, are slightly more stringent values than those that would have been obtained if the numbers of degrees of freedom had been employed in the calculations.

Application of the sampling limits $\pm 2S_{YL}$ to the line of relation, as illustrated graphically in Figs. 2 and 3, leaves no doubt as to the essential truth of the

TABLE X

CORRESPONDING VALUES OF AVERAGES OF SAMPLES OF THREE DRAWN FROM THE SUBGROUPS OF SAMPLES PACKED BY COMPANY *A*

Cannery	Variate	Average of three							
I	pH	6.33	6.22	6.21	6.28	6.30	6.31	6.39	6.33
	O. R.*	4.00	2.00	2.00	3.00	3.33	3.00	2.67	2.67
I	pH	6.26	6.35	6.34	6.27	6.36	6.27	6.28	6.31
	O. R.*	2.67	3.00	2.67	2.33	2.67	2.67	2.67	2.00
I	pH	6.32	6.34	6.33	6.42	6.27	6.45		
	O. R.*	2.00	3.00	2.33	3.00	3.00	4.00		

* *O.R. = Odour rating.*

TABLE XI

CORRESPONDING VALUES OF AVERAGES OF SAMPLES OF THREE DRAWN FROM THE SUBGROUPS OF SAMPLES PACKED BY COMPANY *B*

Cannery	Variate	Average of three							
II	pH	6.31	6.28	6.31	6.32	6.30	6.33	6.31	
	O. R.*	2.33	2.00	2.00	2.33	2.00	3.33	3.33	
II	pH	6.28	6.29	6.36	6.28	6.25	6.35		
	O. R.*	3.67	3.67	4.00	3.33	3.67	3.33		
III	pH	6.34	6.38	6.45	6.41	6.55	6.39	6.37	6.44
	O. R.*	2.67	3.00	3.00	4.67	5.00	4.67	3.33	3.67

* *O.R. = Odour rating.*

hypothesis tested, for only one point (Fig. 3) is found outside these limits, and this only slightly beyond the limit. If, as seems reasonable to believe, the distribution of averages of three is approximately normal, we should expect to find, once in 20 times in the long run, a point slightly outside these limits, and this is precisely what is shown in Fig. 3.

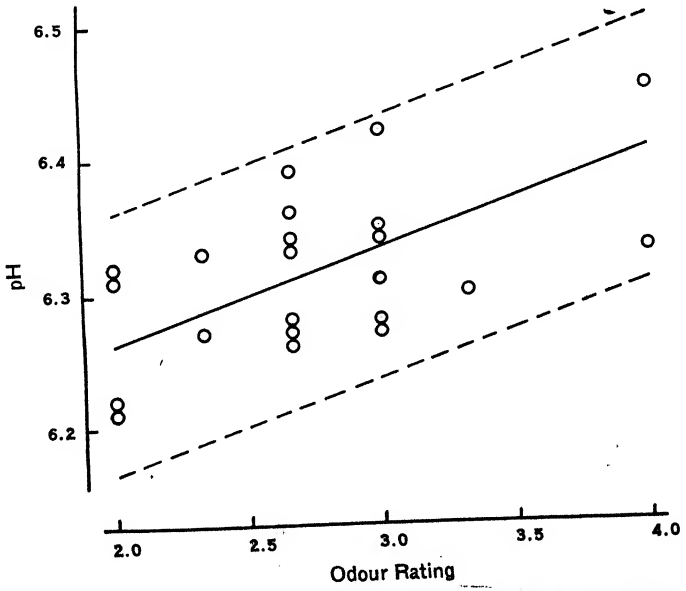


FIG. 2. *Graphical illustration of test of linearity of relation between pH and odour rating by means of sampling limits corresponding to* $\pm 2S_{YL}$ *set on averages of three. Company A.*

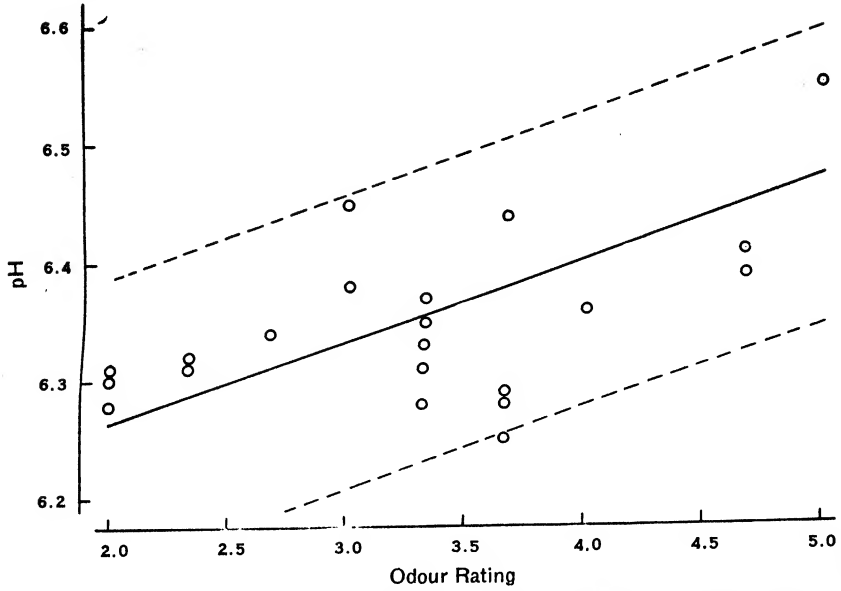


FIG. 3. *Graphical illustration of test of linearity of relation between pH and odour rating by means of sampling limits corresponding to* $\pm 2S_{YL}$ *set on averages of three. Company B.*

Conclusion

The foregoing results thus indicate that the pH value of fish muscle tissue, or aqueous liquors derived therefrom, is closely associated with three fundamental criteria of freshness, namely, with chemical, bacteriological, and organoleptic criteria. Over the range 6.15 to 6.65 the relation between average pH value and average odour rating in large samples is apparently linear. If, therefore, this relation is confirmed by samples packed in canneries located in other districts, it would afford a simple objective means of grading canned chum salmon with respect to freshness, since, by adjusting the size of the sample taken for examination, the examiner's rating for freshness could evidently be determined from the pH values of the individual samples to any desired level of significance. This follows immediately from the fact that, if $\bar{y}_n$ is the average pH of a sample of n, then the standard deviation of $\bar{y}_n$ is $\sigma_n = \frac{\sigma_e}{\sqrt{n}}$. Consequently, if the level of significance is P corresponding to $t\sigma$, the probability is $1 - P$ that the quality of the parcel lies within the limits $\bar{y}_n \pm t\sigma_n$, and hence, that the examiner's rating for the parcel lies within the limits $\frac{\bar{y}_n - \bar{y}}{m} + \bar{x} \pm \frac{t\sigma_n}{m}$, where $\bar{y}$ and $\bar{x}$ are the respective means of the composite distribution.

Before such a method can be safely utilized for practical grading purposes, however, it will be necessary to carry out further work to decide such questions as whether or not the above relation is applicable to samples packed in

other districts, the suitability of this particular examiner's ratings, the ranges in pH and odour rating for other species, and the contribution of the biological condition of the salmon at the time of packing to the examiner's ratings.

Acknowledgment

The writers express their sincere thanks to Miss Margaret Gordon for her valuable assistance in carrying out tabulations and many of the calculations in the preparation of this paper.

References

1. BRITTON, H. T. S. Hydrogen ions. D. Van Nostrand Company, Inc., New York. 1931.
2. CHARNLEY, F. Can. J. Research, A, 19 : 139-151. 1941.
3. FISHER, R. A. Statistical methods for research workers. 5th ed. rev. and enl. Oliver and Boyd, Ltd., Edinburgh and London. 1934.
4. PEARSON, E. S. J. Roy. Stat. Soc. (n.s.) 96(1) : 21-60; Discussion, 60-75. 1933.
5. SHEWHART, W. A. Economic control of quality of manufactured product. D. Van Nostrand Company, Inc., New York. 1931.
6. STANSBY, M. E. and GRIFFITHS, F. P. Ind. Eng. Chem. 27(12) : 1452-1458. 1935.
7. VAN SLYKE, D. D. J. Biol. Chem. 52(2) : 525-570. 1922.

MARINE MACROPLANKTON FROM THE CANADIAN EASTERN ARCTIC

I. AMPHIPODA AND SCHIZOPODA[1]

By M. J. Dunbar[2]

Abstract

Twenty-four amphipod species (one a new species), three euphausiids, and two mysids are recorded from the coastal water of the Canadian eastern Arctic. Most of the records are new.

The list is representative of a high arctic plankton, giving no evidence of the intrusion of Atlantic water. This is in agreement with the hydrographic observations made, and with available hydrographic data from other sources.

The plankton is contrasted with that found in 1936 in Disko Bay, west Greenland, where there appears to be an upwelling of mixed Arctic and Atlantic water. The difference between the plankton of the two sides of Baffin Bay suggests the possibility of distinguishing water of Lancaster Sound (Canadian polar water) from that of west Greenland by means of their planktonic fauna.

Introduction

There is a great scarcity of plankton material collected from the Canadian Arctic. The expeditions that have worked in this region, notably the Second Norwegian Arctic Expedition of 1898 and the Canadian Arctic Expedition of 1913–1918, have been concerned primarily with other work. The Hudson Bay Expedition of 1921 collected some material from Hudson Bay itself. Danish expeditions have collected plankton in Baffin Bay and Davis Strait, but the emphasis has been on the Greenland rather than the Canadian side. The assumption that species found in waters around Greenland, Spitsbergen, and the Siberian coast are circumpolar in distribution has included the American Arctic with the rest and has made its investigation, as far as the marine life is concerned, appear unnecessary. But to ignore one of the two big outlets from the polar basin, namely the Lancaster Sound current, and to derive knowledge of polar water in large part from the east Greenland outlet only, is not justifiable. To understand the movements of polar water, both within the polar basin and outside it, movements vitally important both to the northern fisheries and to the existence of the Eskimos, it is necessary to know the plankton of the American Arctic, and its investigation therefore becomes a matter of some importance. The present study, a survey of the plankton of the coastal water of the Canadian eastern Arctic, has brought to light several points of interest, both biological and oceanographic, which will be emphasized as they arise in this account. The collections were made in the summers of 1939 and 1940 while the author was a member of the Canadian Eastern Arctic Patrols of those years.

[1] *Manuscript received September 5, 1941.*

Contribution from the Department of Zoology, McGill University, Montreal, Que., with financial aid from the National Research Council, Ottawa, Canada.

[2] *National Research Council Student.*

The Area

The collecting stations are shown in the map (Fig. 1). The majority of the work was done at Lake Harbour, Frobisher Bay, and Clyde River. It will be seen that most of the stations are on the Baffin Island coasts.

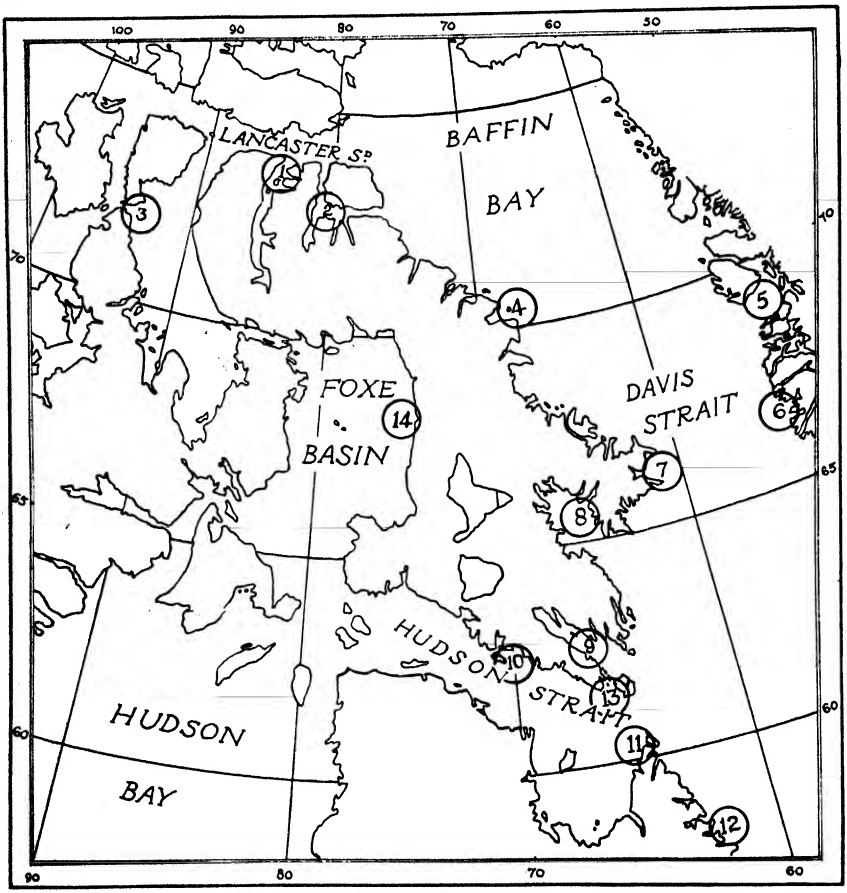


FIG. 1. *Map of the area investigated. 1. Arctic Bay; 2. Pond Inlet; 3. Fort Ross; 4. Clyde River; 5. Disko Bay; 6. Holsteinsborg; 7. Cape Walsingham; 8. Pangnirtung (Cumberland Gulf); 9. Frobisher Bay; 10. Lake Harbour; 11. Port Burwell; 12. Hebron; 13. Gabriel Strait; 14. Hantzsch River.*

Methods

A motor boat was used at all stations except at Clyde River where, for the two weeks' work, the only boat available was a sailing whaleboat, which did not prove entirely satisfactory. Two nets were used: a one-metre stramin ring-trawl and a silk net of 29 meshes per cm. During the two seasons the following hauls were made.

Stramin:

63 horizontal hauls of 30 min. each, at depths varying from 40 metres to the surface; two oblique hauls from a depth of 75 metres.

5 horizontal hauls of 40 min. each.

6 horizontal hauls of from 7 to 15 min. each.

20 current hauls, using the tidal current to sift the water through the net; time of hauling usually 30 min.

8 vertical hauls, from various depths, all under 50 metres, to the surface.

Silk:

11 horizontal surface hauls of 15 min. each.

3 horizontal surface hauls of 30 min. each.

2 current hauls, surface.

5 vertical hauls, from depths less than 60 metres.

Thus the ordinary lower limit of operations was at 40 metres. At this depth the plankton was found to be scarce in the coastal water, the bulk occurring higher up.

Immediately on being hauled into the boat the catches were concentrated with a small conical concentrating net, and preserved in 10% formalin in sea water, in 4-oz. screw-top bottles. In the laboratory, the preservative was changed to 5% formalin in sea water, and a little borax was added.

The Hydrography of the Area

The submarine ridge between Cape Walsingham in Baffin Island and Holsteinsborg in west Greenland seems to prevent the passage northward of the bulk of the Atlantic water found in the deeper layers south of the ridge. Reporting on the work of the U.S. Coast Guard expeditions in the *Marion* and the *General Greene*, Smith, Soule, and Mosby (35) calculate that the water in Baffin Bay is derived in approximately equal quantities from the Lancaster Sound current and the west Greenland current. The west Greenland current is shown to be a mixture of east Greenland polar water and Atlantic water, and therefore some Atlantic water must be carried over the Holsteinsborg ridge. North of the ridge, however, it has lost its original physical characters, and its influence in the north and west of Baffin Bay has been assumed to be very small, an assumption that is supported by the results of the *Godthaab* expedition (17, 24) and of the present work. The changes that are occurring in the water off west Greenland will be discussed later; it seems that there is a significant difference between the water of Disko Bay and Baffin Island. For present purposes it is enough to point out that the available hydrographic data seem to indicate that water around Baffin Island is of purely arctic character. From what is known of the arctic indicator species, the collection here described points to the same conclusion.

In 1940, water samples and temperatures were taken at several stations with a reversing-type water bottle and a Richter thermometer. Standard

Copenhagen sea water was obtained from Woods Hole, through the kindness of Dr. Iselin. The results are shown in Table I. The low salinities at Clyde River are due to the presence of the river itself, a circumstance unfortunately unavoidable. The observations were made in order to find out whether there was any trace of non-Arctic water demonstrable hydrographically, within the limits of the scale of the work. It is clear from the table that there is no evidence for the presence of foreign water. These figures agree closely with those of Hachey (8) for the waters of Hudson Strait.

TABLE I

HYDROGRAPHIC DATA, 1940

Date	Station	Depth, m.	Temperature, °C.	Salinity, ‰
28/7	Hudson Strait, 20 miles from land, southeast of Big Island	75	−0.81	33.62
28/7	Lake Harbour anchorage, high tide	0	1.56	30.31
		10	0.33	31.07
		25	−0.13	31.65
		46	−1.18	32.93
31/7	Lake Harbour, six miles from anchorage	0	3.68	30.74
		10	−0.12	31.78
		25	−0.54	32.28
		50	−0.66	32.58
		75	−0.72	32.62
6/8	Frobisher Bay	0	1.15	30.69
		10	0.10	32.34
		25	−0.04	32.34
		50	−0.06	32.24
		75	−0.13	32.34
14/8	Lake Harbour, 10 miles from anchorage	0	2.15	31.67
		10	0.21	32.28
23/8	Lake Harbour, six miles from anchorage	0	0.85	32.50
		10	0.22	32.58
		25	0.38	32.60
		50	0.35	32.72
11/9	Clyde River	50	−1.39	—
17/9	Clyde River	0	0.52	27.83
		10	0.60	28.04
		25	0.55	29.88
		50	−1.32	32.50
22/9	Pangnirtung anchorage	0	1.66	29.61
		10	1.09	31.41
		25	0.75	31.71
		50	0.62	32.03

The Plankton

In giving the authorities for the specific names of the animals listed below, the advice and avowed practice of Osgood (21) has been followed in omitting the parentheses around the authorities for forms in which the genus has been changed since the original naming of the species.

Under "records from the American Arctic", records have been included from Alaska to the Newfoundland Labrador, excluding Greenland. Geographically, Greenland is in the American Arctic, but in its history, both political and scientific, it belongs to Europe, and there are good reasons for excluding it from oceanographic arctic America. Most of the species named in this report have been recorded from Greenland waters.

Order AMPHIPODA

Suborder HYPERIIDEA

Family HYPERIIDAE

Hyperia galba Montagu. Taken at Lake Harbour and Gabriel Strait. Three specimens.

Records from the American Arctic: Collinson Point, (Alaska (32); Hudson Bay (33)).

Further distribution: North Atlantic, Spitsbergen, Novaya Zemlya, Murman coast, Greenland, Arctic Ocean; also known from the north Pacific (27, 30).

Hyperia medusarum Müller. Taken at Lake Harbour. Five specimens.

Records from the American Arctic: Hudson Bay (32), Cumberland Gulf (36), Labrador (37), and Point Barrow, Alaska (18).

Further distribution: Arctic, north Atlantic southward to about the 55th parallel, and north Pacific (30); California coast (13); commonest in depths between 200 and 1800 metres (30).

Hyperia spingera Bovallius. Taken at Lake Harbour and Clyde River. Two specimens. At Lake Harbour, found in the stomach of a ringed seal.

This species is not often collected, and Stephensen (43) has omitted it from the list in Fauna Arctica. Schellenberg (30) gives as its distribution: "Spitsbergen, north coast of Norway, south coast of England, western Ireland, and the Labrador current."

Hyperia sp. There were several specimens of this genus that were too small for identification of the species. These were taken at Fort Ross, Arctic Bay, Gabriel Strait, and Clyde River.

Hyperoche medusarum Kröyer. Taken at Burwell, Lake Harbour, Arctic Bay, Pangnirtung, Gabriel Strait, and Clyde River. Fifteen specimens. More common than the *Hyperia* species.

In the American Arctic, this species has been recorded from Alaska by the Canadian Arctic Expedition (32), from Labrador (51), and from Newfoundland (6, 23).

Further distribution: West Greenland (9, 44), Gulf of St. Lawrence (34), north Atlantic and adjacent Arctic (30); "possibly circumpolar" (44).

Themisto libellula Mandt

Syn. *Euthemisto libellula* Mandt

Taken at all stations, from Hebron to Fort Ross. Several thousand specimens.

In the American Arctic, *T. libellula* has been found at Bernard Harbour and the Dolphin and Union Strait, both free and in the stomachs of *Phoca hispida* Schreber and *Salvelinus malma* Walbaum (32); Hudson Bay (33), and Point Barrow (18).

All workers have commented on the abundance of this form in the Arctic. Tesch (50) says of it that it "shows a marked preference for arctic water". Stephensen (41) records several hundred small specimens from surface water in east Greenland; describing the *Godthaab* expedition material (44), he considers it to be a typically Arctic species. Russell (26) from data supplied by Stephensen, has it in the list of cold water species, but he has omitted it from the list of Arctic indicator species, since it seems able to live, for a time at least, in warmer water. Schellenberg (30) gives its distribution as "in all arctic seas, southward to about 43° N., surface to 2500 metres."

T. libellula is without doubt one of the most important organisms in the Arctic, in any habitat, terrestrial or aquatic. It has been shown (5) to be the main food of the ringed seal during the summer at least, and probably for a large part of the year. The full investigation of its biology would be a profitable undertaking. It is comparable, in its position in the economy of the waters, to the key species of the Antarctic, *Euphausia superba* Dana, and seems to take the place, in high latitudes, of the "Krill" (*Thysanoessa inermis* Kröyer and *T. raschii* M. Sars) which form the bulk of the diet of the whalebone whales in the Atlantic.

In the fjord waters, *Themisto* was found to have a vertical optimum noticeably higher in the water than the rest of the zooplankton. It may be that it feeds largely on the phytoplankton, or on the ostracods that were found in large numbers at the surface. At all events, it showed no tendency to sink lower in the water during the daytime, and it was frequently seen and caught in great quantity right at the surface of the sea, even on the brightest of days. It is supposed that this tolerance to bright sunlight is due to its deep purple pigmentation. The pteropod, *Limacina helicina* Phipps, which like *Themisto* is darkly coloured, is also found close to the surface in great profuseness.

Themisto abyssorum Boeck

Syn. *Euthemisto abyssorum* Boeck

Parathemisto oblivia Kröyer

Taken at Lake Harbour and Hebron. Nine specimens.

American Arctic records: several stations of the Canadian Arctic Expedition on the north Alaskan coast (32); Humboldt Bay, Popof Island, Alaska (14).

Further distribution: Gulf of St. Lawrence (34 and 23), Esquiman Channel (22), and west of Greenland (16); "In all northern seas, in the Atlantic south to about 45° N." (30).

Suborder GAMMARIDEA

Family LYSIANASSIDAE

Anonyx nugax Phipps. Taken only once, at Gabriel Strait.

Recorded in the American Arctic from Dolphin and Union Strait (32), and from Port Burwell (37).

Widely distributed in arctic and boreal seas, most commonly found in the littoral zone.

Pseudalibrotus littoralis Kröyer. Taken at Fort Ross and at Pangnirtung. Eight specimens identified with certainty.

Recorded in the American Arctic from Collinson Point, Alaska, Bernard Harbour, and Dolphin and Union Strait (32), Point Barrow (18), and Hudson Bay (33). Specimens collected by Mr. T. H. Manning off Hantzsch River, west Baffin Island, and sent to the author for identification, belong to this species.

"Circumpolar arctic. 0–1000 m., benthonic; often free-swimming in large swarms." (30).

Pseudalibrotus glacialis Sars. Taken at Hebron, Lake Harbour, Fort Ross, Pond Inlet, Arctic Bay, Pangnirtung, Gabriel Strait, Frobisher Bay, and Clyde River. One hundred and eighteen specimens. Also eight specimens from stomachs of ringed seals at Lake Harbour.

American Arctic records: Collinson Point and Point Barrow (32).

Distribution: arctic, pelagic. Recorded from northern Spitsbergen and the Siberian Sea, and from east and west Greenland (30, 28, 44, and 45); also from north Norway (47).

Pseudalibrotus nanseni Sars. Taken at Lake Harbour, Gabriel Strait, and Frobisher Bay. Six specimens. Also two specimens from a ringed seal's stomach at Lake Harbour, one a very large female measuring 21 mm.

Recorded from Point Barrow (32).

Further distribution much the same as for *P. glacialis*.

Pseudalibrotus sp. Specimens too small and immature to identify with certainty were taken at Lake Harbour and Fort Ross.

The genus *Pseudalibrotus* has given difficulty to taxonomists for some time. The three species listed here, together with *P. birulai* Gurjanowa (7), are all that have so far been reported from the Arctic. *P. glacialis* and *P. nanseni* were described by Sars (28) from material of the Norwegian North Polar Expedition, 1893–1896. In 1917 Stephensen (40) referred to *P. nanseni*, specimens that were immature and these, owing to uncertainty as to the correctness of the determination, he figured in great detail. Later, in working out the material of the Ingolf Expedition, he settled the matter of the determination of the species (41). In identification he relied only on the notch on the inner ramus of the second uropod in *P. littoralis*, and on

the shape of the sixth segment of the second gnathopod in *P. nanseni* and *P. glacialis* (*P. birulai* had not been described). He stated "The characters used in this key seem to me to be the best for determination. They are much better than the coxal plate of pereiopod 1, and the length of the distal part of pereiopod 7 which may vary considerably. Specimens smaller than 4–5 mm. can scarcely be determined."

Sars (28) did not describe the male of *P. glacialis*. Certain differences were recorded in the male by Stephensen (41): "Antennae a little longer. Uropoda almost as in *P. nanseni*, and uropod 3 has natatory setae, not 'without any marginal setae' (Sars). The telson has one pair of apical spines." In the material from Baffin Island, these characters were certainly found in the males, but, with the exception of the long antennae, they were found in many of the females as well. Thus of 12 mature females from Pangnirtung, seven had apical spines on the telson, and eight had one or two long setae on the third uropods, though never as many as in the male. In one male from Pangnirtung, there were no spines on the telson.

Specimens of *P. glacialis*, both male and female, taken in the eastern Arctic, reach somewhat beyond the size limit given by Sars (28). Sars found no specimens of this species above 9 mm. in length. Many of the females in the present collection are longer than this, and there are males measuring as much as 13.5 mm. from rostrum to telson.

With these reservations, the existing descriptions of the species of *Pseudalibrotus* are adequate. Using Stephensen's (41) system, there is no difficulty in distinguishing the species in specimens larger than 5 mm.

Family METOPIDAE

Metopa longirama n. sp.* One specimen from Clyde River; a female, with brood lamellae well developed. (Figs. 2 to 11).

General body form close to *M. alderi* Bate; last three pereiopods rather more slender. Antennae long, a little more than half the length of the body. Cephalon roundly produced at the sides, anterolateral corners sharp. Eyes medium, rounded. Coxal plates similar to *M. alderi*. Mouth parts typical of the genus; inner plates of the maxillipeds very small. Finger of first gnathopod long, about two-thirds as long as the sixth joint; fourth joint bearing a row of fine bristles. Second gnathopod, sixth joint ovoid in shape, and large, almost twice as long as the head; two strong spines at proximal end of grasping edge; no emargination. Pereiopods normal, the backward processes on the fourth and fifth joints of pereiopods 4 and 5 (6 and 7 according to Schellenberg's (30) system) not strongly developed. Pleopods very long, the rami two and one-half times as long as the basal portion. Uropods typical of the genus, third uropod with one ramus only. Telson smoothly rounded, with no spinules. Length 4 mm. (female).

Family ACANTHONOTOSOMIDAE

Acanthonotosoma inflatum Kröyer. Two specimens, taken at Lake Harbour.

Recorded in the American Arctic from Totness Road, Baffin Island (44), Labrador (37), and Collinson Point, Alaska (32).

Known from the Gulf of St. Lawrence (34), Greenland (42), Spitsbergen (29 and 19). "A true arctic, probably circumpolar littoral species" (44).

Family OEDICEROTIDAE

Westwoodilla brevicalcar Goës

Syn. *Halimedon brevicalcar* Goës, in Sars (27)

One specimen taken at Gabriel Strait.

This is the first record of this species from the American Arctic. It was recorded for the first time on the Atlantic coast of Canada by Shoemaker (34), from the collection of the Cheticamp expedition in the Gulf of St. Lawrence.

Distribution: Arctic ocean, Iceland, Greenland (9), and Spitsbergen (29).

Family CALLIOPIIDAE

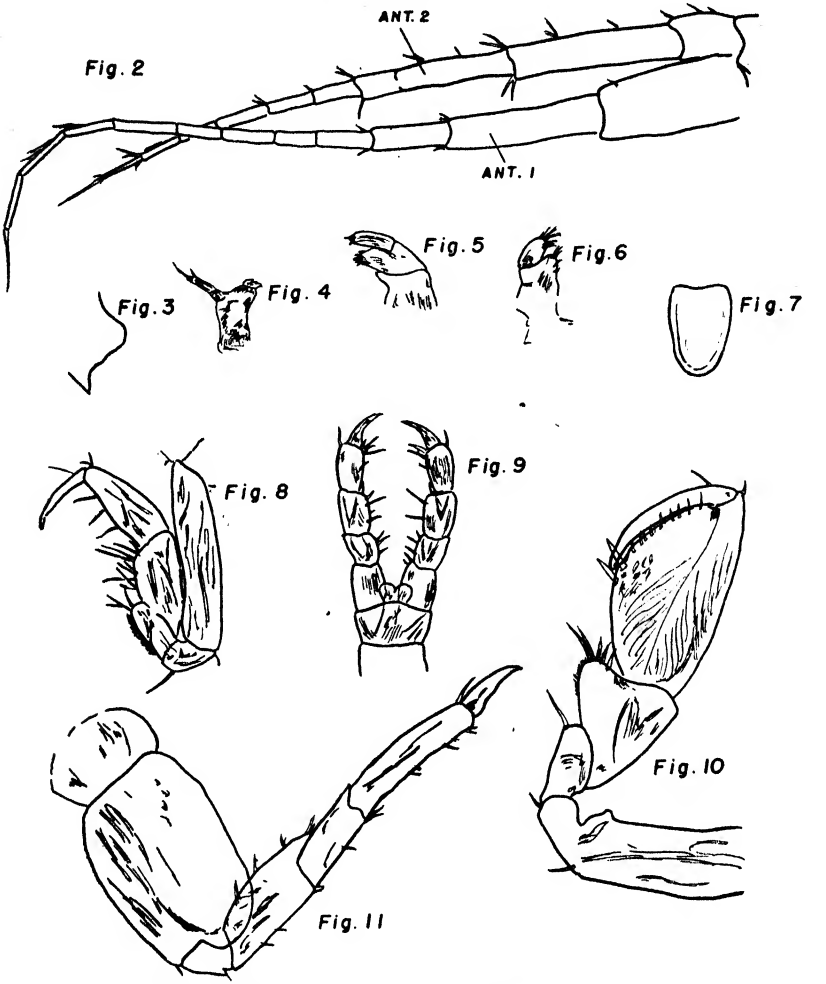
Calliopius laeviusculus Kröyer. One specimen from Lake Harbour. The specimen agrees with Sars's (27) description and figure, except that the last epimer is somewhat more smoothly rounded, and the legs are not quite so hairy. It is, however, an immature specimen.

American Arctic records: Labrador (37), three stations in Alaskan and western Arctic waters (32), and Hudson Bay (33).

Further distribution: Newfoundland (6), Straits of Belle Isle (22), Gulf of St. Lawrence (34); Greenland, Spitsbergen, Franz Joseph Land, Norway, British Isles, north Pacific (30).

---

* *The unique specimen and type is in the author's collection, at present in the Department of Zoology, McGill University, Montreal, Que.*



FIGS. 2 TO 11. *Metopa longirama n. sp.* FIG. 2. *Antennae;* FIG. 3. *Cephalon, lateral view;* FIG. 4. *Mandible;* FIG. 5. *First maxilla;* FIG. 6. *Second maxilla;* FIG. 7. *Telson;* FIG. 8. *First gnathopod;* FIG. 9. *Maxillipeds;* FIG. 10. *Second gnathopod;* FIG. 11. *Last thoracic leg.*

Apherusa megalops Buchholtz

Syn. *Paramphithoë megalops* Buchholtz (3)
Halirages megalops Stebbing (38)
Apherusa megalops Shoemaker (32, 33, and 34)
Halirages megalops Stephensen (42)

This is not the same species as the *Apherusa megalops* Sars (27) which was first described by Sars as *Halirages megalops*. Shoemaker (34) proposed the name *Apherusa sarsii* for *A. megalops* Sars.

Taken at Hebron, Lake Harbour, Gabriel Strait, and Fort Ross. Twenty-three specimens.

Recorded in the American Arctic from Hudson Bay (33) and Bernard Harbour (32). This latter record, resulting from the Canadian Arctic Expedition, was the first in North America. Up till then the species was not known outside of Greenland waters. Later it was recorded by Shoemaker (34) from the Gulf of St. Lawrence.

Apherusa glacialis H. J. Hansen. Taken at Hebron, Lake Harbour, Gabriel Strait, Clyde River, and Fort Ross. Found also in the stomach of a ringed seal at Clyde River. Altogether 42 specimens.

Recorded from Point Barrow, Bernard Harbour, and Harrison Bay (Alaska) (32). A circumpolar Arctic species, known also from the Gulf of St. Lawrence and Newfoundland. Pelagic.

Family PONTOGENEIDAE

Pontogeneia inermis Kröyer. One specimen taken at Gabriel Strait.

Recorded from Labrador (37), Hudson Bay (33), Bernard Harbour (32), Gulf of St. Lawrence (34), and Newfoundland (6).

Further distribution: Bay of Fundy, New England coast, Greenland coasts, British Isles, Siberian Sea.

Family PLEUSTIDAE

Pleustes panoplus Kröyer. One immature specimen from Lake Harbour. The dorsal carina is not very distinct, and the triangular prominence on the telson is not developed. Otherwise it agrees with the descriptions of Stebbing (38) and Sars (27). Sars mentions that the strong tuberculation of the body is far less distinct in immature specimens than in the adults.

Recorded from Port Burwell (37), and from the Gulf of St. Lawrence (34). Widely distributed in the arctic and subarctic littoral zones.

Family GAMMARIDAE

Gammarus locusta Linn. Taken at Lake Harbour, Pangnirtung, Pond Inlet, Arctic Bay, and Fort Ross. Also found in stomachs of ringed seals at Lake Harbour and Clyde River. Twenty specimens.

This species, widely distributed along arctic and northern temperate coasts, has been recorded from Point Barrow (18), Cumberland Gulf (36), Labrador (37), Hudson Bay (33), and from Collinson Point to Dolphin and Union Strait (32).

Gammaracanthus loricatus Sabine. Found in the stomach of a ringed seal at Lake Harbour. One specimen.

Recorded from Point Barrow (18), Collinson Point and Bernard Harbour (32), and Hudson Bay (33). Fairly common in arctic seas, but not found further south. It is interesting that no specimen of this species was found by the Cheticamp expedition in the Gulf of St. Lawrence, where so many Arctic forms have been recorded.

Family JASSIDAE

Ischyrocerus megacheir Boeck. One specimen from Lake Harbour.

Recorded from Hudson Bay (33) and the Gulf of St. Lawrence (34).

Further distribution: Greenland, Iceland, Spitsbergen, White Sea, Skagerrak, Bering Sea.

Ischyrocerus anguipes Kröyer. Nine specimens from Fort Ross, all ovigerous females.

Recorded from Bernard Harbour (32). Widely distributed in the arctic and adjacent seas (44).

Family PODOCERIDAE

Dulichia tuberculata Boeck

Syn. *Dulichia curticauda* Sars (27)

One specimen, a male, from Hebron.

The second gnathopods are not quite so large as in Sars's (27) figure, and the basal tooth is not so finely tapered.

An Arctic littoral species (27).

Dulichia sp. One female from Fort Ross; a damaged specimen, which cannot be determined with certainty.

Suborder CAPRELLIDEA

Family CAPRELLIDAE

Caprella septentrionalis Kröyer. Five specimens from Hebron.

Recorded from Cumberland Gulf (36), Labrador (37), and Hudson Bay (33).

Widely distributed: known "from the waters west of Greenland and the New England States to Novaya Zemlya and Denmark; Japan" (44).

Order EUPHAUSIACEA

Family EUPHAUSIIDAE

Thysanoessa inermis Kröyer

Syn. *Rhoda inermis* Kröyer

One specimen only, from the stomach of a ringed seal at Clyde River.

Recorded in the waters of the American Arctic from Point Barrow, by the Canadian Arctic Expedition (31); also found by the same expedition in the stomach of *Phoca hispida*. Stephensen (46) describes this species as "widely distributed, ... but most frequent in arctic waters". According to Robertson (25) it is the most important food of the cod off Bear Island. It is the most common euphausiid in the Arctic, but was never taken in the nets during the present work. It must be restricted, in the Baffin Island region, to the open water, if it is common at all in that region; it does not occur in the fjords to any great extent. More work is required on the Canadian distribution of this and the following species. On the present evidence, it is impossible to agree with Stephensen that *Thysanoessa inermis* is most frequent in Arctic waters.

This species has been shown to be the staple food of various whales (1). It is found over the whole of the north Atlantic, and has been recorded from the north Pacific (11). In eastern Canada, it has been recorded from several stations in the Gulf of St. Lawrence, Straits of Belle Isle, and Newfoundland waters (49).

Thysanoessa raschii M. Sars

Syn. *Rhoda raschii* M. Sars

Taken once in the net, at night, in the open water at the mouth of Frobisher Bay. Also found in seal stomachs at Lake Harbour. Altogether three specimens.

The distribution of this species is much the same as for *T. inermis*. In Arctic America, it has been found at Collinson Point, and from stomachs of *Phoca hispida* and *Salvelinus malma* at Bernard Harbour (31).

Meganyctiphanes norvegica M. Sars. Taken only once, in the stomach of a specimen of *Phoca hispida* at Lake Harbour.

No record has been found of this species from Arctic America. Stephensen (46) says of it: "A north Atlantic species, found from east America and Greenland to Siberia and the Mediterranean. It is rarely found in arctic waters." He records it from west Greenland up to 63° 19′ N. and from the coast of King Christian X's Land in east Greenland (45). It has also been found in Disko Bay, approximately 69° latitude, in west Greenland by Hartley and Dunbar (unpublished data). It is known from the Gulf of St. Lawrence, Nova Scotia waters, and Massachusetts Bay (10).

Order MYSIDACEA

Family MYSIDAE

Mysis oculata Fabricius. Taken at Fort Ross, Hebron, Gabriel Strait, and Pangnirtung; often found in stomachs of *Phoca hispida* at Lake Harbour, Clyde River, and Frobisher Bay (5). Seventy specimens taken in the net, many more from seals.

An Arctic, circumpolar, littoral species. It is nekonic rather than benthonic, being more often taken in nets than in dredges. It has been recorded from all shores washed by Arctic water, including Labrador (37), Bay of Islands, Newfoundland (49), Collinson Point, Bernard Harbour, Dolphin and Union Strait, and Cape Kellett (Banks Island) (31); Spitsbergen (2), Greenland (46, etc.), and also from the west of Canada (48).

Mysis mixta Lilljeborg

Syn. *Mictheimysis mixta* Lilljeborg

Found in stomachs of *Phoca hispida* at Lake Harbour and Clyde River, and in the stomach of *P. groenlandica* Erxleben at Frobisher Bay. Nine specimens.

An arctic–boreal littoral species, not hitherto recorded from Arctic America.

Discussion

Not all the species listed above are truly planktonic. *Acanthonotosoma*, *Gammarus*, *Ischyrocerus*, *Dulichia*, and *Caprella* were all obtained in shallow water, with the net close to the bottom, and are to be regarded as littoral or benthonic forms rather than planktonic.

The list is clearly representative of a high arctic fauna, "high arctic" being used to describe areas in the north where the influence of Atlantic water, or of Pacific water, is negligible or absent. The number of species is small, a character of polar regions, and there is no member that belongs strictly to Atlantic water. *Themisto libellula*, *Pseudalibrotus littoralis*, *P. nanseni*, *P. glacialis*, *Gammaracanthus loricatus*, *Westwoodilla brevicalcar*, *Acanthonotosoma inflatum*, and *Apherusa glacialis* have all been taken as typically arctic species, and, at least if found in the upper layers, as indicative of Arctic water (39). Russell (26) has no amphipod in his list of Arctic indicators, on the grounds that the arctic species are able to survive for some time in water of higher temperature. Stephensen (44) gives four species of amphipods as typical pelagic Arctic forms, namely *Pseudalibrotus nanseni*, *P. glacialis*, *Apherusa glacialis*, and *Themisto libellula*. The remaining species in the present collection are eurythermal forms found in both Arctic and Atlantic areas; individuals from both areas are morphologically identical, species for species, though it has been suggested by Orton (20) that they may be physiologically separate.

A very interesting aspect of the list is the contrast it offers to the analogous fauna in Disko Bay in 1936. Disko Bay (Fig. 1) is in west Greenland, just below the 70th parallel of latitude, and about level with Arctic Harbour and Home Bay, south of Clyde River, in Baffin Island. The distance between the two stations is not great, but the difference in the plankton is remarkable. The place of *Themisto libellula* in the Baffin Island water was taken in Disko Bay by the euphausiids *Thysanoessa inermis* and *T. raschii*. *T. libellula* was not abundant in Disko Bay, and in fact the species *T. abyssorum* was considerably more common than *libellula*. *Meganyctiphanes norvegica* was encountered fairly often, and the mysid *Boreomysis nobilis* G. O. Sars was very common (4).

The "key industry" species in the two regions are *Themisto libellula* in Baffin Island waters and *Thysanoessa* in Disko Bay. Of these, *Themisto* is Arctic in distribution and *Thysanoessa* widely distributed in the Atlantic, if not predominantly so. Of the other Disko Bay forms, *Meganyctiphanes norvegica*, according to Stephensen (46), is a north Atlantic species rarely taken in the Arctic; *Themisto abyssorum*, rare in Canadian Arctic waters and common in Disko Bay, is widely distributed in the Atlantic; *Boreomysis nobilis* is an Arctic species (52), found in deep water, and its presence in Disko Bay and absence in the eastern Arctic coastal water is therefore interesting. Lastly, certain specimens of the chaetognath, *Sagitta elegans* Verrill, which were found to have matured in Disko Bay at a smaller size than the bulk of the population (4), suggest the presence of non-Arctic water.

There is thus evidence for two suppositions; first, that there is an upwelling of mixed Atlantic and Arctic water in Disko Bay, bringing into the bay both Atlantic forms and deep-water Arctic forms, and second, that the influence of Atlantic water at the coast of Baffin Island is very small. The surprising scarcity of *Thysanoessa* and absence of *Boreomysis* in the Baffin Island collections can have two possible explanations; either that these forms do not belong to Lancaster Sound polar water, or that they keep to the deeper water in the Baffin area. Further work on a larger scale than the present will settle this exceedingly interesting point.

This interpretation of the Baffin and Disko Bay amphipod and schizopod plankton is confirmed by the water itself. The water samples taken in Disko Bay were somewhat warmer and contained more salt than the Baffin Island samples shown in Table I. Negative temperatures were met only once, inside the Jakobshavn Icefjord, where negative temperatures would not be surprising. At all other stations the temperatures from 10 to 100 metres ranged from 0.16° to 2.55° C., as against −1.39° to 1.0° C. in Baffin water. Salinities were a little higher in Disko Bay. Thus the salinity at 50 metres varied between 33.00 °/₀₀ and 33.60 °/₀₀ (12), whereas Table I shows salinities of from 32.03 to 32.72 °/₀₀ at 50 metres (excluding the abnormally low value obtained at Clyde River).

Jensen (15) has reviewed the marked warming in climate, both atmospheric and marine, which has occurred in recent decades in west Greenland and eastward. Sea temperatures, up to and including 1936, have been getting higher, and various animals of boreal habit have been extending their ranges to the north, together with a corresponding tendency on the part of the arctic forms to retreat still further north, a circumstance that has made possible, in west Greenland, the establishment of a commercial cod fishery of considerable importance. The plankton found in Disko Bay in 1936 is thus in agreement with this general invasion from the south. In the faunal comparison made above, the plankton of Disko Bay in 1936 is compared with that of the Baffin Island coasts in 1939 and 1940. To the best of the author's belief, there has not been published, in the last five years, any account of a change in the plankton of Disko Bay but, in a postscript to his paper, Jensen gives data that indicate such a change is not impossible. It seems that from 1937 onwards to the date of the paper (1939) temperatures have been falling again, and the fjord cod (*Gadus ogac* Richardson) has again become common in southwest Greenland. It is not known whether the water of east Baffin Island has been behaving in the same way, but the presence of the Lancaster Sound current would render the contingency remote. Such possibilities as this, however, are among the many good reasons from the establishment and maintenance of permanent bases in the Canadian Arctic for the routine continuance of the observation of the sea, both hydrographic and biological.

Whether the comparison made here between the plankton of the two sides of Baffin Bay is considered in three dimensions or four, certain conclusions can be drawn as to the possible use of the species as indicators in the Baffin-

Labrador–Newfoundland area. In the upper layers, *Themisto libellula*, *Pseudalibrotus nanseni*, *P. glacialis*, and *Apherusa glacialis* are reliable indicators of Arctic water. *Boreomysis nobilis*, if found in the upper coastal water, may be taken as indicative of upwelling Arctic water, possibly of Greenland origin. *Thysanoessa inermis* and *Thysanoessa raschii*, being found in both Arctic and Atlantic water, cannot be used to distinguish between the two, but their extreme scarcity at the Baffin Island coast demands further investigation; it is possible that they may be useful to distinguish between Lancaster Sound and west Greenland water.

References

1. ALLEN, G. M. Mem. Boston Soc. Nat. Hist. 8(2) : 105-322. 1916.
2. BJÖRCK, W. Kgl. Svenska Vetenskapsakad. Handl. (ny följd) 54(6) : 1-10. 1916.
3. BUCHHOLTZ, R. Zweite deutsche Nordpol-farht, 1869-1870, 2 : 262-399. 1874.
4. DUNBAR, M. J. J. Anim. Ecol. 9(2) : 215-226. 1940.
5. DUNBAR, M. J. Can. J. Research, D, 19 : 150-155. 1941.
6. FROST, N. Newfoundland Dept. Nat. Res., Research Bull. 3 : 1-10. 1936.
7. GURJANOWA, E. Zool. Anz. 81(11 and 12) : 309-317. 1929.
8. HACHEY, H. B. Contrib. Can. Biol. Fisheries (n.s.), 7(9) : 91-118. 1931.
9. HANSEN, H. J. Vidensk. Medd. Naturh. Foren. 39 : 5-226. 1887.
10. HANSEN, H. J. Danish Ingolf Expedition, 3(2) : 1-120. 1908.
11. HANSEN, H. J. Proc. U.S. Natl. Museum, 48 : 59-114. 1915.
12. HARTLEY, C. H. and DUNBAR, M. J. Sears Foundation, J. Mar. Research, 1(4) : 305-311. 1938.
13. HOLMES, S. J. Proc. U.S. Natl. Museum, 35 : 489-543. 1909.
14. HOLMES, S. J. Harriman Alaska Expedition, 1899, 10 : 231-246. 1910.
15. JENSEN, S. Kgl. Danske Videnskab. Selskab. Biol. Medd. 14(8) : 1-75. 1939.
16. JESPERSEN, P. Medd. Grønland, 64(4) : 101-160. 1923.
17. KIILERICH, A. B. Medd. Grønland, 78(5) : 1-149. 1939.
18. MURDOCH, J. Intern. Polar Expedition Point Barrow, Alaska, Pt. IV(5) : 136-176. 1885.
19. OLDEVIG, H. Kgl. Svenska Vetenskapsakad. Handl. (ny följd) 54(8) : 1-56. 1917.
20. ORTON, J. H. J. Marine Biol. Assoc. United Kingdom (n.s.), 12(2) : 339-366. 1920.
21. OSGOOD, W. H. Science, 89 : 9-11. 1939.
22. PINHEY, K. F. Contrib. Can. Biol. Fisheries (n.s.), 3(6) : 179-233. 1927.
23. PINHEY, K. F. Contrib. Can. Biol. Fisheries (n.s.), 3(13) : 331-346. 1927.
24. RIIS-CARSTENSEN, E. Medd. Grønland, 78(3) : 1-101. 1936.
25. ROBERTSON, J. A. Rapp. proc.-verb. conseil intern. exploration mer, 81 : 115-139. 1932.
26. RUSSELL, F. S. Rapp. proc.-verb. conseil intern. exploration mer, 95 : 3-30. 1936.
27. SARS, G. O. An account of the Crustacea of Norway. Vol. I. Christiana. 1895.
28. SARS, G. O. Norwegian North Polar Expedition, 1893–1896, Sci. Results 1(5) : 1-141. 1900.
29. SCHELLENBERG, A. Mitt. Zool. Museum Berlin, 11 : 193-231. 1924.
30. SCHELLENBERG, A. Nord. Plankton, 3 : 589-722. 1927.
31. SCHMITT, W. L. Can. Arctic Expedition, 1913-1918, 7(B) : 1-8. 1919.
32. SHOEMAKER, C. R. Can. Arctic Expedition, 1913–1918, 7(E) : 1-30. 1920.
33. SHOEMAKER, C. R. Contrib. Can. Biol. Fisheries (n.s.), 3(1) : 1-11. 1926.
34. SHOEMAKER, C. R. Contrib. Can. Biol. Fisheries (n.s.), 5(10) : 219-359. 1930.
35. SMITH, E. H., SOULE, F. M., and MOSBY, O. U.S. Treasury Dept., Coast Guard, Bull. 19. 1937.
36. SMITH, S. I. U.S. Natl. Museum, Bull. 15 : 139-140. 1879.
37. SMITH, S. I. Proc. U.S. Natl. Museum, 6 : 223-232. 1884.
38. STEBBING, T. R. R. Tierreich, 21 : I–XXXIX, 1-806. 1906.
39. STEPHENSEN, K. Medd. Grønland, 45(11) : 501-630. 1912.
40. STEPHENSEN, K. Medd. Grønland, 53(3) : 229-378. 1917.

41. STEPHENSEN, K. Danish Ingolf Expedition, 3(8) : 1-100. 1923.
42. STEPHENSEN, K. Danish Ingolf Expedition, 3(11) : 179-290. 1931.
43. STEPHENSEN, K. Fauna Arctica, 6(4) : 343-378. 1932.
44. STEPHENSEN, K. Medd. Grønland, 79(7) : 1-88. 1933.
45. STEPHENSEN, K. Medd. Grønland, 104(15) : 1-12. 1933.
46. STEPHENSEN, K. Medd. Grønland, 79(9) : 1-20. 1933.
47. STEPHENSEN, K. Tromsø Museums Skrifter, 3(1) : 1-140. 1935.
48. TATTERSALL, W. M. Contrib. Can. Biol. Fisheries (n.s.), 8(15) : 181-205. 1933.
49. TATTERSALL, W. M. J. Fisheries Research Board Can. 4(4) : 281-286. 1939.
50. TESCH, J. J. Bull. trimestriel conseil intern. exploration mer, 2 : 176-193. 1911.
51. WHITEAVES, J. F. Catalogue of the marine invertebrates of eastern Canada. Geological Survey of Canada, Ottawa. (No. 772). 1901.
52. ZIMMER, C. Nord. Plankton, 3 : 1-178. 1909.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. FEBRUARY, 1942 NUMBER 2

THE EFFECT OF ETHYL ALCOHOL AND TRICRESOL ON THE VITAMIN A CONTENT OF THE BLOOD AND LIVER OF THE CHICKEN[1]

W. A. MAW[2] AND N. NIKOLAICZUK[3]

Abstract

That preservatives in perishable biological extracts may specifically alter physiological function cannot be precluded in the analysis of experimental results. Whereas 15% ethyl alcohol is shown to significantly lower both vitamin A and carotene concentrations of the liver in growing chickens, little effect is manifest upon similar blood constituents; 0.1% and 1.0% levels of tricresol were without effect upon either vitamin A or carotene in blood or liver, but prompted a pronounced increase in total mean body weight of 30% above the control; alcohol slightly depressed gains. Both preservative materials under study markedly reduced the amount of carotenoids in the shank skins. The extent of such effects in herein reported.

Introduction

The storage of biological extracts for future use necessitates the addition of preservatives such as dilute ethyl alcohol and tricresol. Ethyl alcohol when added to yield a concentration of 15% and tricresol at a level of 0.1% have been found to possess excellent preservative qualities. The effect of these admixtures and their relation to the problem under study cannot be disregarded in the analyses of the results.

Studies at Macdonald College (2, 4) demonstrated that the metabolic fraction of the anterior pituitary (622*c*), in which 15% ethyl alcohol was used as a preservative, gave an increased vitamin A content in the liver of the growing chicken. It was observed also that alcohol tended to decrease this effect. These findings were further substantiated in later work (3) in which the carotenoid contents of the rations were under study.

Data published by Clausen (1) suggest an effect of ethyl alcohol on vitamin A metabolism in dogs and the authors' experiments prove a similar effect upon chickens.

[1] *Manuscript received July 10, 1941.*

Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Q. e., Canada, with assistance from the National Research Council, Ottawa, Canada. Macdonald College Journal Series No. 164.

[2] *Assistant Professor of Poultry Husbandry and Head of the Department of Poultry Husbandry.*

[3] *Lecturer, Department of Poultry Husbandry.*

Experimental

Forty Leghorn cockerels, 10 weeks in age, were randomized for body weight and allotted to four groups of 10 birds each. All lots were fed a dry mash and water ad libitum. Lot 1, the control group, was injected intramuscularly with ½ cc. distilled water twice daily. Lots 2, 3, and 4 received similar injections of 15% ethyl alcohol, 0.1% and 1.0% tricresol, respectively.

The period of treatment was 21 days, after which all birds were killed and blood samples were taken by severing the jugular vein posterior to the angle of the lower mandible. The blood was allowed to clot and the serum was collected for vitamin A and carotene analyses. The livers were removed for immediate analyses after the birds were bled. The shank skins were also analysed for carotenoid pigment content.

Results

Data presented in Table I reveal that ethyl alcohol has a marked influence on the amount of vitamin A in the liver and the carotene of both the liver and shank skins, as shown by the appreciably lower values for these constituents when compared with both control and tricresol lots. The blood carotene and vitamin A do not appear to be significantly affected by the alcohol, but some reduction in the former is observed. The carotene and vitamin A contents of the liver are significantly lower than those of the control group, which would suggest that mechanisms of withdrawal are stimulated by the continuous presence of alcohol or its effect in conjunction with normal metabolic processes.

TABLE I

THE EFFECT OF ETHYL ALCOHOL AND TRICRESOL UPON THE MOBILIZATION OF VITAMIN A POTENT SUBSTANCES IN THE LIVER AND BLOOD OF GROWING CHICKENS

(Summary of 10 individuals per lot)

Treatment*	Gain in body weight, gm.	Liver weight, gm.	Percentage of total body weight	Liver carotene, mg./gm.	Liver vitamin A, mg./gm.	Blood carotene, mg./100 cc. serum	Blood vitamin A, mg./100 cc. serum	Shank skin carotenoids, mg./gm.
Water	296	18.29	1.77	1.03	15.80	0.725	0.072	0.027
Ethyl alcohol	277	18.55	1.95	0.55	11.80	0.682	0.077	0.013
Tricresol, 0.1%	397	24.24	2.20	1.29	14.50	0.711	0.067	0.019
Tricresol, 1.0%	383	21.13	2.04	1.04	14.30	0.791	0.065	—

* *Injection intramuscular, ½ cc. twice daily.*

Tricresol at 0.1% and 1.0% levels has no marked effect upon the vitamin A and carotene content of the liver, nor upon the quantity of these constituents in the blood serum.

Whereas alcohol depressed growth as measured by mean gain in body weight, 277 gm. as compared with 296 gm. for the control, tricresol at both

levels prompted greater total gains, approximately 30% higher than the control. The specific significance of the growth promoting properties of tricresol is obscure on the basis of the data recorded in this experiment.

Liver weights in the tricresol groups are significantly greater than in the control group. This is, undoubtedly, the effect of tricresol treatment as demonstrated by the increased proportion of liver weight to body weight, namely, 2.20% and 2.04% as compared with 1.77% for the control and 1.95% for the alcohol group.

The carotenoid content of the shank skins is greatly reduced by alcohol and tricresol injections.

Acknowledgments

The authors wish to acknowledge the assistance of Messrs. T. M. MacIntyre, F. P. Griffin, and W. E. Parker and the co-operation of the Department of Chemistry in the analytical work.

References

1. Clausen, S. W., Baum, W. S., McCoord, A. B., Rydeen, J. O., and Breese, B. B. Science, 91 : 318-319. 1940.
2. Maw, W. A., McFarlane, W. D., Parker, W. E., Stuart, J. A., and Collip, J. B. Abstract. Poultry Sci. 18 : 407. 1939.
3. Maw, W. A., Nikolaiczuk, N., MacIntyre, T. M., Parker, W. E., Griffin, F. P., McFarlane, W. D., and Collip, J. B. Abstract. Poultry Sci. 19 : 355-356. 1940.
4. Maw, W. A., Stuart, J. A., and Collip, J. B. Am. J. Physiol. 126 (3) : P582. 1939.

A NEW SPECIES OF *ONCHIDIOPSIS* FROM BAFFIN LAND

By Henry D. Russell

Abstract

A detailed description of a new species of gastropod mollusc, *Onchidiopsis kingmaruensis*, is given. The specimen on which the description is based is the second of this genus to have been found in the western Atlantic region and was collected at Lake Harbour Fiord, Baffin Land, by Dr. John Oughton of the Royal Ontario Museum, Toronto, Ont. The species is compared with *O. corys* Balch.

While on a trip to Labrador and Baffin Land during 1939, Dr. John Oughton of the Royal Ontario Museum, Toronto, Ont., collected a single specimen of *Onchidiopsis* which he has most kindly sent to the author for identification. The specimen was obtained on August 4 in 30 fathoms of water at Lake Harbour Fiord, Baffin Land. After a critical examination the author believes the specimen to be a new and valid species; a description and comparison with *O. corys* Balch follows below.

Onchidiopsis kingmaruensis n. sp.*

(Figs. 1 to 9)

The holotype is No. 17,260 in the collection of the Royal Ontario Museum of Zoology, Toronto, Ont.

Size

The length of the specimen preserved in alcohol is 24 mm.; the width, 15 mm.; and the height, 17 mm.

Colour

Foot, head, and notaeum are uniformly grayish-yellow, suggestive of a water blister; eye spots black.

General Form (Fig. 4)

The notaeum is wrinkled over its entire surface suggesting the contours of the human brain. These wrinkles become more pronounced as they near the ventral border. The notaeum rises at an angle of 25° from the anterior towards the posterior end and covers the large visceral hump.

Below the notaeum the foot of moderate size bearing dorsolaterally ruffled edges is plainly visible. Its blunt anterior angles and notched anterior edge extend slightly beyond the origin of the tentacles. Posteriorly, it terminates in a blunt point just before reaching the posterior edge of the notaeum. It turns sharply upward at its posterior extremity for about one-fourth its length as described by Balch (1, p. 475).

[1] *Manuscript received September 18, 1941.*
Contribution from the Museum of Comparative Zoology, Cambridge, Mass., U.S.A.

[2] *Assistant Curator of Molluscs.*

* *The name "Kingmaru" is the Eskimo word for Lake Harbour.*

The thick obtusely pointed tentacles extend for half their length beyond the anterior edge of the foot. Laterally the circular black eyes are borne at a point about halfway between the origin and tip of these tentacles.

The large penis situated on the right side of the neck and dorsal to the right tentacle is not visible beneath the anterior portion of the notaeum.

Notaeum

The notaeum is fleshy and deeply wrinkled except over the dorsal part of the visceral mass where the wrinkles become less well defined. Slightly to the left of the median line anteriorly is the ill defined inspiratory cleft and on the right at a point two-fifths of the distance between the anterior and posterior extremities of the animal is located the far more distinct expiratory cleft. Both of these, however, resemble folds in the notaeum rather than actual clefts.

Shell (Fig. 3)

The shell is chitinous, very thin, and situated immediately beneath the notaeum. It appears as a transparent horny film. There is a small central point at the otherwise straight-edged anterior end and the posterior edge is rounded. The sculpture consists of concentric growth lines with the nucleus at the anterior end. The shell is highly arched both from the sides and ends towards the centre. It measures 19 mm. long, 10 mm. wide, and 6 mm. high at the centre, its highest point. It is somewhat firmer than that described by Balch (1, p. 471) since when held by forceps it will support itself against gravity in air. This is true only if it is held by the posterior end.

Gill cavity and branchial complex

On opening the arched branchial chamber situated on the dorsal side of the neck and between it and the visor-like anterior fold of the notaeum which covers the head, the osphradial and branchial laminae are immediately visible. As the observer holds the animal in the normal position for crawling, with the notaeum uppermost, the head facing him and the foot below, the gill appears on the left and the osphradium on the right of the cavity. The gill (Fig. 5) consists of 22 simply pointed, cream coloured laminae that extend into the branchial chamber from its thin dorsal wall. The osphradium consists of a double series of 11 leaflets supported by a central rachis and extending into the branchial chamber from the dorsal wall. The leaflets are unequal in shape, those on the left being larger and more pointed than the semicircular ones situated on the right side of the rachis. Both sets of leaflets possess a black pigment colouring their dorsal edge. Except for this black pigment the osphradium is cream coloured and the rachis is white.

Tentacles and eyes

The tentacles arise from the anterior of the dorsoventrally sloping head. They are bluntly pointed and about half as wide as long. For half their length they are faintly wrinkled and uniform in width. Then they swell slightly and become more deeply wrinkled and gradually narrow to a blunt

point. The eyes (Fig. 8) are small, round, black, and laterally situated on the tentacles at the point where the swelling takes place.

Foot (Figs. 5, 8, 9)

The foot in contraction is about three times as long as wide. Anteriorly it terminates in short, blunt, lateral angles or auricles. The anterior edge is slightly notched at the centre and possesses a deep horizontal groove which extends from the tip of one auricle to the tip of the other. Posteriorly the foot terminates in a bluntly rounded point which turns sharply upward against the notaeum. On the dorsal surface of the foot and extending posteriorly from the base of the anterior angles is a distinct, irregularly ruffled area 2 mm. wide. It is slightly narrower at its anterior and posterior ends than at the central portion. This ruffle is composed of dorsoventral folds that are somewhat higher than the dorsal surface of the foot (Fig. 9). Where the posterior dorsal surface between the lateral ruffles presses against the notaeum it possesses a triangular thickening or pad. The plantar surface of the foot is cream coloured, but the dorsal surface is tinged with gray.

Radula

The radula (Fig. 1) is taenioglossate and has the formula

$$\frac{1}{2}, \frac{5+1+5}{1}, \frac{5+2+1+1+5}{1}, \frac{5+1+5}{1}, \frac{1}{2}.$$

The uncinal hooks (Fig. 1A) are long, non-dentate, smooth, and sharp. The claw-like part of the older teeth is about as long as the base. That of younger teeth is about twice as long as the base and is longer, narrower, and more sharply pointed than is that of the older teeth (Fig. 2).

The elliptical reflection of the laterals (Fig. 1B) bears four to five small bluntly pointed cusps on either side of the long sharply pointed median cusp. The base of the laterals is bluntly rounded, more slender and triangular in shape than is that of the median teeth.

The older median teeth are mushroom-shaped with a very broad and short "stipe". The elliptical reflection is flat across its anterior surface or slightly depressed towards the centre. It bears five to six small bluntly pointed cusps on either side of the much longer and more sharply pointed central one. The sides of the pillar or "stipe" are concave and enlarge into an obtusely rounded basal portion. The younger medians (Fig. 1C) bear two or three small sharply pointed denticles at the base of and lateral to the large central cusp. Usually there is one small cusp on one side and two on the other, making a total of 13 to 14 cusps. The presence of these small cusps on the younger teeth and the lack of them on the older ones seems to be the only difference between the medians of varying ages in the radula.

Other Characters

The mouth appears merely as a horizontal slit between and slightly ventral to the tentacles. No rostrum was observed.

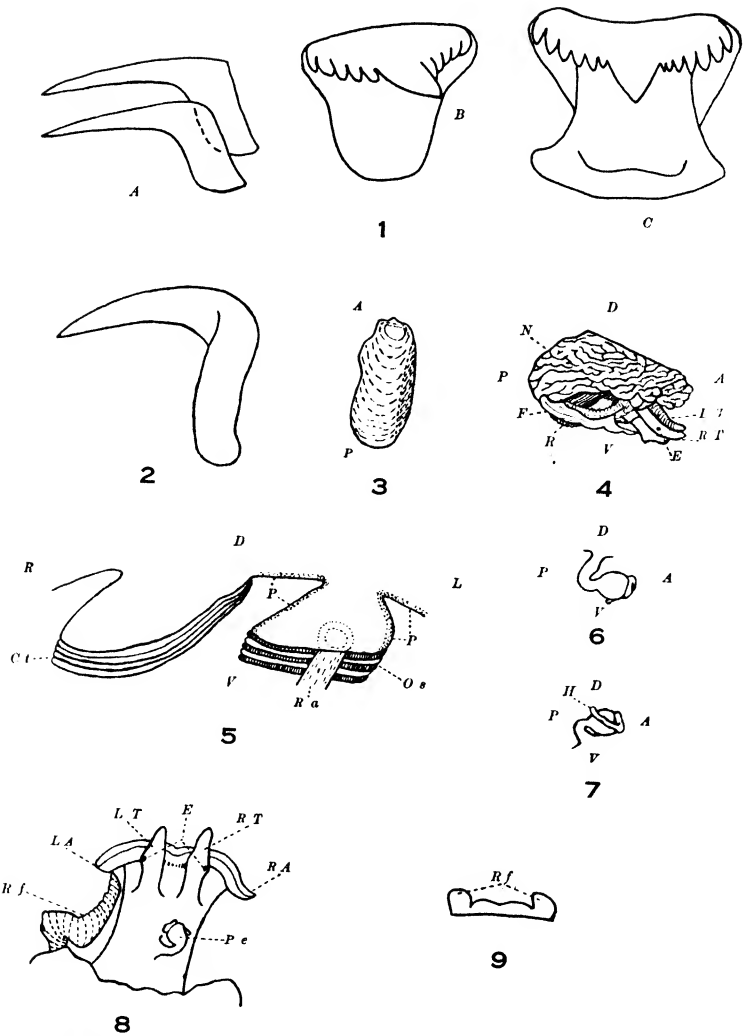


FIG. 1. *Left half of single transverse row of teeth from younger portion of radula. A, uncinal teeth; B, lateral tooth; C, median tooth (greatly enlarged).*

FIG. 2. *Uncinal tooth from older portion of radula (greatly enlarged).*

FIG. 3. *Dorsal aspect of shell. A, anterior; P, posterior. Natural size.*

FIG. 4. *Right lateral view of animal. A, anterior, P, posterior end; D, dorsal, V, ventral surface; N, notaeum; F, foot; R, ruffle, LT, left tentacle; RT, right tentacle; E, eye. Natural size.*

FIG. 5. *Branchial complex. R, right side; L, left side; D, dorsal, V, ventral surface; P, pigmented area; Ra, rachis of osphradium; Os, osphradium; Ct, ctenidium (greatly enlarged).*

FIG. 6. *Penis. A, anterior, P, posterior end; D, dorsal, V, ventral rod of penis (greatly enlarged).*

FIG. 7. *View of under side of penis showing the rod, H; D, dorsal, V, ventral surface; A, anterior, P, posterior end (greatly enlarged).*

FIG. 8. *Dorsal aspect of head. RA, right anterior angle of foot; LA, left anterior angle of foot; RT, right tentacle; LT, left tentacle; E, eyes; Rf, ruffle of foot; Pe, penis. ×3.*

FIG. 9. *Transverse section through posterior portion of foot, showing dorsolateral ruffles, Rf. ×4.*

The fragile jaws were not examined, probably having been destroyed during the removal of the buccal mass. It is probable, however, that they are similar to those described by Bergh (2, Pl. 3, Fig. 29, *a* to *b*) and by Balch (1, Pl. 22, Fig. 4).

About midway between the right tentacle and the branchial chamber is the large penis (Figs. 6, 7, 8). This arises slightly to the left and dorsal to the right tentacle. As it proceeds laterally, it narrows and bends almost directly back upon itself swelling suddenly to about twice its original width and resembling a clenched fist. This is capped by a single rounded coil of tissue which after making one complete turn continues as a rod along the side of the swollen portion of the penis and between it and the dorsolateral wall of the neck (Fig. 7H). The penis is somewhat wrinkled over its entire surface.

In Table I, the characters described above are summarized and compared with those of *O. corys*.

TABLE I

COMPARISON OF THE CHARACTERS OF *O. corys* BALCH AND *O. kingmaruensis* RUSSELL

Characters	*O. corys*	*O. kingmaruensis*
1. Colour	In formol:— ground colour purplish-brown with darker markings on the lower sides; colour pattern gives the effect of 12 ill defined blotches of the ground colour.	In alcohol:— uniformly yellowish-gray; no colour pattern.
2. Notaeum	Smooth on top and sides, wrinkled or strongly folded and vesiculate elsewhere.	Wrinkled over its entire surface, but much more strongly so on the sides than immediately over the visceral mass.
3. Shell	Very thin; does not resist the action of gravity in air; posterior–inferior portion folds into the posterior–superior portion.	Thin; can resist the action of gravity in air as above mentioned; shell more elongate than that of *O. corys;* no infolding of the posterior–inferior portion as in *O. corys.*
4. Branchial complex	Osphradium consists of a double row of bilobed leaflets, approximately equal in shape.	Osphradium consists of a double row of leaflets unequal in shape as above related.
5. Foot	Posterior; projects beyond the notaeum; dorsolateral edge smooth.	Posterior; does not project beyond the notaeum; dorsolateral edge deeply ruffled.
6. Radula	Median tooth with 6 to 10 lateral denticles; laterals with a median cusp flanked by seven denticles on the external and five on the internal side; uncini with smoothly arched claw.	Older median tooth with five to eight lateral denticles on each side; younger median with eight to nine lateral denticles on each side; laterals with four to five denticulations on either side of the median cusp; uncini with smooth arched claw.

Discussion

Of the four known species and subspecies of *Onchidiopsis* given by Balch (1, p. 477) only one, *O. corys* Balch is neoboreal or western Atlantic in distribution. Thus *O. kingmaruensis* is the second specimen and species of this genus to be reported from this side of the Atlantic, since Balch's specimen was a unique. There are so many character differences between *O. corys* and *O. kingmaruensis* that the author feels no hesitancy in believing this latter to be a new species.

References

1. BALCH, F. N. Proc. U.S. Natl. Museum, 38 : 469-484. 1911.
2. BERGH, R. Kgl. Danske Videnskab. Selskab Skrifter, Naturvidenskab. math. Afdel. (Ser. 5) 3 : 239-350. 1853.

1942

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. MARCH, 1942 NUMBER 3

PRESERVATION OF EGGS. I.

TREATMENTS FOR MAINTAINING QUALITY IN SHELL EGGS AT ORDINARY TEMPERATURES[1]

BY F. T. ROSSER[2], W. H. WHITE[3], A. H. WOODCOCK[4], AND D. A. FLETCHER[5]

Abstract

Cultural studies demonstrated the fungicidal value of urea and dimethylol urea and the effectiveness of the vapours of certain volatile solids as mould growth inhibitors. A method for preparing sterile artificial eggs for mould studies was developed.

Investigations on 21 treatments for preserving eggs at high temperatures indicated that the best results would be obtained by the following procedure: conditioning with carbon dioxide to lower the pH, treating shell surfaces with a disinfectant to reduce contamination followed by effective sealing, preferably with a substance having properties that would prevent both growth and entrance of contaminants during subsequent storage. Dimethylol urea was the most effective growth inhibitor for micro-organisms and vaseline proved to be the best sealing agent. Satisfactory results were obtained by dipping eggs in polyvinyl alcohol treated with dimethylol urea and by packing oil dipped eggs in moisture resistant bags.

Introduction

Shipments of shell eggs from Canada to Great Britain are often carried in unrefrigerated stowage. Under war conditions the extended transport period together with the increased volume exported has resulted in appreciable quantities of eggs arriving at their destination in poor condition. Although loss of quality from a number of causes has been encountered, the growth of micro-organisms both within the egg and on the outer shell has been the principal cause of deterioration. Certain treatments such as oiling, gas storage, and impregnation of the fillers and flats with mould inhibiting agents have been advocated as effective for extending the storage life of eggs at cold storage temperatures but little is known regarding their efficacy at higher temperatures. The present laboratory studies were made to obtain information on the value of some of the old, and certain new, processing treatments for the preservation of egg quality during storage under conditions of high temperature and humidity.

[1] *Manuscript received January 28, 1942.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 1042 and as Paper No. 75 of the Canadian Committee on Storage and Transport of Food.

[2] *Mycologist, National Research Laboratories.*

[3] *Biochemist, Food Storage Investigations, National Research Laboratories.*

[4] *Biophysicist, Food Storage Investigations, National Research Laboratories.*

[5] *Agricultural Assistant, Poultry Marketing and Inspection Services, Department of Agriculture, Ottawa.*

It must be recognized that high temperature during the shipping period may not be the only cause of deterioration in commercial consignments. Eggs may be subjected to rapid temperature changes during loading or discharging with the result that condensate may form on the shell. Such conditions are known to bring about the penetration of micro-organisms through the shell and to accelerate the growth of exterior moulds. In some of these experiments, therefore, an attempt was also made to assess the value of a treatment for preventing deterioration attributable to temperature fluctuations and condensate formation.

The experiments to be described fall into two classes: (i) preliminary tests of a given material or treatment using cultures or sterile artificial eggs; and (ii) storage tests using from 3 to 15 eggs per treatment. The inhibitors studied may be classified as *contact*, those substances that act only when in contact with micro-organisms, and *distant*, those whose vapours are sufficient to hinder growth. Both types of inhibitors were tested by an appropriate culture procedure. However, since the efficacy of treatments involving dipping could not be assessed in this way, a method was also developed for preparing sterile artificial eggs to study effects of treatments on specific moulds when the physical barrier of the shell was taken into consideration. Although the results obtained to date are limited, the application of this procedure promises to yield useful information.

Cultural Studies on Mouldicides

Methods

In all culture experiments, Petri plates containing potato dextrose agar were inoculated with small amounts of scrapings from a mould culture. In the contact treatments the chemical was combined with the medium before

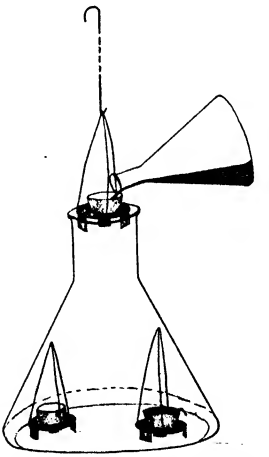


Fig. 1. *Diagram of apparatus used in preparation of sterile artificial eggs.*

sterilization. Cultures subjected to vapour were placed in the upper portion of desiccators in the lower section of which were four 2-in. squares of blotting paper previously soaked in the chemical solution. Cultures receiving reduced vapour treatments were placed in desiccators each of which was filled with mixtures of air and air saturated with the vapour. Colony diameter was used as the criterion for the mouldicidal value of a given treatment. Each treatment was replicated six times in the vapour experiments and three in the contact and reduced vapour tests.

In the preparation of sterile artificial eggs the rounded end of an egg shell was cut off, care being taken not to crack the shell. The shell membranes were removed and the cleaned shell placed open side up in a small metal holder (Fig. 1). Three such holders were lowered into a Fernbach flask and sterilized in the autoclave after which they were raised in turn into the neck of the flask and filled with potato dextrose agar to about one-eighth inch from the shell rim. When this had solidified a layer of sterile paraffin wax was run over it and for convenience in handling a small bent metal strip was inserted into the wax at the edge of the shell. Recognized bacteriological technique was followed throughout.

TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF UREA AND DIMETHYLOL UREA ON THE GROWTH OF SOME COMMON EGG MOULDS

Treatment	Conc., % wt./vol.	Colony diameter, mm.								
		Penicillium citrinum 18 days	*Aspergillus niger* 6 days	*Chaetomium globosum* 8 days	*Mucor racemosus* 6 days	*Penicillium citrinum* 8 days	*Aspergillus niger* 10 days	*Chaetomium globosum* 8 days	*Mucor racemosus* 3 days	*Penicillium citrinum* 12 days
Control		52.4	79	21	13.3	15	83	97	96.3	57.5
Urea	0.25	45.3	—	—	—	—	—	—	—	—
	0.5	28.6	—	—	—	—	—	—	—	—
	1.0	14.8	—	—	—	—	—	—	—	—
	1.5	13.1	—	—	—	—	—	—	—	—
	2.0	9.3	—	—	—	—	—	—	—	—
	2.5	7.6	—	—	—	—	—	—	—	—
	3.0	7.8	—	—	—	—	—	—	—	—
Dimethylol urea	0.008	—	—	—	—	—	79.0	98.0	92.0	52.2
	0.016	—	—	—	—	—	80.5	26.6	88.2	49.6
	0.031	—	—	—	—	—	71.1	0	66.2	47.8
	0.063	—	50.3	0	9.1	0	0	0	0	39.6
	0.125	—	0	0	0	0	0	0	0	0
	0.25	0	0	0	0	0	0	0	0	0
	0.5	0	0	0	0	0	—	—	—	—
	1.0	0	0	0	0	0	—	—	—	—
	1.5	0	—	—	—	—	—	—	—	—
	2.0	0	—	—	—	—	—	—	—	—
	2.5	0	—	—	—	—	—	—	—	—
	3.0	0	—	—	—	—	—	—	—	—

CONTACT AND VAPOUR MOULD GROWTH INHIBITORS

In a previous investigation (4) it was found that small concentrations of urea had marked inhibitory effect on the growth of certain parasitic fungi. It was thought that dimethylol urea might also act as a mouldicide. To test this assumption media containing various concentrations of urea and dimethylol urea were inoculated with four species of common egg moulds. The results are shown in Table I. Dimethylol urea appears to be a very effective mouldicide, since no growth occurred on media containing more than 0.063% of the chemical. In contrast urea in concentrations up to 3% retarded but did not prevent growth of *Penicillium citrinum* Thom.

The vapour of the tri-, tetra-, and pentachlorophenates has been found effective in inhibiting mould growth on eggs when the subliming source was close to the egg (3). In view of this it was suggested that the vapour of a compound known as DuPont Experimental Product IN-3102[1] might also act as a mould inhibitor. Laboratory tests were therefore undertaken to compare the mould growth inhibiting powers of IN-3102 with sodium pentachlorophenate. A common egg mould, *Penicillium citrinum* was grown in saturated atmospheres of vapours of both these chemicals and in several concentrations of IN-3102 vapour.

The results, presented in Tables II and III, show that a saturated atmosphere of the vapour of IN-3102 had a marked inhibitory effect on the growth of the mould whereas that of sodium pentachlorophenate had no apparent effect. Colonies affected by IN-3102 were deeply furrowed and wrinkled with

TABLE II

EFFECT OF VAPOURS OF SODIUM PENTACHLOROPHENATE AND DUPONT EXPERIMENTAL PRODUCT IN-3102 ON THE GROWTH OF *Penicillium citrinum* THOM.

Treatment	Incubation period, days									
	2	4	6	8	10	12	14	16	18	20
	Colony diameter, mm.; incubation at 21° C.									
Control	7.0	14.2	22.0	29.8	38.6	48.5	58.1	67.4		
Sodium pentachlorophenate	7.7	14.7	22.8	30.0	40.4	50.0	58.1	69.0		
IN-3102	4.9	6.8	7.8	8.3	8.8	9.5	10.6	10.7		
	Colony diameter, mm.; incubation at 4.4° C.									
Control		3.4		5.1		6.9		9.1		9.9
Sodium pentachlorophenate		3.4		5.3		7.2		8.7		9.6
IN-3102		3.5		4.5		5.5		6.1		6.5

[1] *Product supplied by Canadian Industries, Limited.*

TABLE III

EFFECT OF REDUCED VAPOUR CONCENTRATIONS OF DUPONT EXPERIMENTAL PRODUCT IN-3102 ON THE GROWTH OF *Penicillium citrinum* THOM.

Initial concentration of IN-3102*	Colony diameter, mm.	
	Incubated 9 days at 21° C.	Incubated 6 weeks at 4.4° C.
0	34.8	20.5
12.5	38.5	16.8
25	41.5	14.8
50	38.6	16.8
100	38.3	12.6
Excess IN-3102 to maintain 100% saturation	14.5	5.0

* *Expressed as percentage of saturated atmosphere.*

a wide, white aureole. It is also evident that concentrations of IN-3102 less than saturated were not effective and in fact, the indications are that small concentrations actually stimulated growth. It should be noted that adsorption of the vapour on the surfaces of the glass containers may have occurred thereby reducing the effective concentrations in all cases except where excess IN-3102 was present.

STERILE ARTIFICIAL EGGS

To study the effect of dipping treatments on external mould growth, the surfaces of 20 sterile artificial eggs were evenly inoculated by spraying from an atomizer a heavy spore suspension of *Penicillium citrinum*. When dry, the eggs were divided into five groups and treated as follows: (1) control (untreated), (2) dipped in oil[1], (3) dipped in oil containing 3% acetic acid, (4) dipped in saturated solution of sodium borate, (5) dipped in a mixture of oil and vaseline. After draining, all eggs were placed in sterile containers. Two from each group were stored immediately at 21° C. and 90% relative humidity; to determine the effect of condensate on mould growth the remainder were chilled at 0° C. for 24 hr. before placing in the storage chamber.

After three weeks' storage mould growth was in evidence on all shells of all five groups. Although no quantitative measurements were made those receiving the sodium borate treatment had the lightest growth and those with the oil and vaseline treatment, the heaviest. There was no noticeable difference between the results for the chilled and unchilled eggs.

[1] *Oil employed in these tests was a low viscosity paraffin oil used commercially for egg dipping.*

The Effectiveness of Polyvinyl Alcohol and Other Treatments in Preserving Shell Eggs

Haines and Moran (2) have demonstrated that a "rough correlation" exists between the porosity of an egg shell and the loss of water by evaporation during storage and that bacteria can be readily drawn through a shell by suction as the egg cools. Since it is generally considered that egg spoilage is caused mainly by organisms that penetrate the shell it would appear that sealing treatments should be at least partially effective in preventing detrimental microbial growth, reducing evaporation, and preserving other attributes of quality. Although there may be practical limitations, combinations of sealing substances with others having mouldicidal and bactericidal properties would appear to be ideal from the standpoint of extending storage life. The following experiments were designed to test various substances and treatments of this sort either alone or in combination.

Material and Methods

Eggs used in storage experiments were all of the first grade. Those obtained for the initial test were bought at local stores; those for subsequent experiments were purchased from egg grading stations, a careful selection of uniform, first quality, white shelled eggs being made.

DuPont polyvinyl alcohol solutions, type *A* (low and high viscosity) and type *B* (medium and high viscosity), were prepared in various concentrations by dissolving with the aid of heat the required number of grams in 100 ml. of water. In all treatments eggs were dipped at room temperature and placed on racks to dry. With a current of air drying could be effected in about five minutes at the lower concentrations of the polyvinyl alcohols. Eggs were conditioned with carbon dioxide by holding for approximately 16 hr. in an atmosphere of the gas. In the vaseline treatment, eggs were dipped in warm liquid vaseline, the excess being wiped off when the egg had cooled.

The storage chamber was set at 21° C. (70° F.) and 90% relative humidity, these conditions being considered satisfactory for accelerating spoilage. In order to assist organisms in penetrating the shell and, if possible, to stimulate the growth of exterior moulds by formation of condensate, some of the eggs were chilled overnight at 40° F. and returned to the warm storage room. This was repeated several times.

After an initial storage period of from four to five weeks the eggs were candled weekly and observations made on their exterior condition. Also, in the third experiment, quality was assessed by pH, white thickness, yolk index, and weight loss measurements. Eggs were broken out on a clean glass plate. White thickness was determined as the height of the white above the plate in approximately the centre of the thick white region. Yolk index was expressed as the height of the yolk above the plate divided by its diameter. Height measurements were taken with a spherometer and diameters with a pair of calipers. The measurements of pH were made with a Beckmann pH

meter. Measurements of pH and white thickness were not made on bad eggs and the yolk index was obtained only for those in which the vitelline membrane remained intact when they were broken out.

As the numbers of eggs tested were small, and the mean percentage of defects following the different treatments varied considerably, the data for the two final experiments were subjected to an inverse sine transformation prior to statistical treatment.

Results

In Table IV are summarized the results of preliminary tests on the preservative value of DuPont polyvinyl alcohols, types *A* and *B*, when used as egg sealing agents. It will be seen that the storage life of all eggs receiving polyvinyl alcohol dips exceeded that of the control and also that the differences in effect between concentrations were not marked. The indications are that the most satisfactory results might be obtained with Type *B* medium viscosity polyvinyl alcohol containing dimethylol urea.

The results of an experiment to study the effect of polyvinyl alcohol treatments on mould growth and air cell size of eggs held both at constant and

TABLE IV

Effect of treatment with various types and concentrations of polyvinyl alcohols on the storage life of eggs held at 21° C. and 90% relative humidity

Treatment	Concentration, %	No. of eggs treated	Proportion showing internal mould or spot rot, %						
			Storage period, wk.						
			4	5	6	7	8	9	10
Control		7	86	100	100	100	100	100	100
DuPont polyvinyl alcohol									
Type *B*:									
Medium viscosity	2	6	50	67	83	83	83	83	83
	6	6	67	67	67	83	83	83	100
	10	4	0	0	0	75	100	100	100
	14	3	0	0	67	67	67	67	100
	14 and 5.6% dimethylol urea	3	0	0	0	0	33	33	100
	18	3	0	0	33	67	67	67	67
High viscosity	2	6	0	33	33	86	100	100	100
	6	5	0	20	20	60	80	80	80
	10	3	0	0	0	33	33	33	100
Type *A*:									
High viscosity	2	3	0	67	100	100	100	100	100
	6	3	0	0	33	67	100	100	100
	10	3	0	0	33	100	100	100	100
	14	3	0	33	33	33	100	100	100
	18	3	0	0	100	100	100	100	100
Low viscosity	18	3	0	33	33	67	67	67	100

TABLE V

THE EFFECT OF POLYVINYL ALCOHOL COATINGS ON THE STORAGE LIFE OF EGGS HELD AT CONSTANT AND ALTERNATING TEMPERATURES*

Treatment	Temperature conditions	Percentage showing visible defects																	
		Internal mould or rot						Marked external mould						Large air cell					
		Storage period, wk.						Storage period, wk.						Storage period, wk.					
		5	6	7	8	9	10	5	6	7	8	9	10	5	6	7	8	9	10
Control	Constant***	83.3	100.0	100.0	100.0	100.0	100.0	16.7	58.3	100.0	100.0	100.0	100.0	0	25.0	100.0	100.0	100.0	100.0
	Alternating†	0	0	16.7	33.3	58.3	83.3	33.3	75.0	75.0	75.0	83.3	91.7	16.7	33.3	33.3	75.0	91.7	100.0
DuPont polyvinyl alcohol, Type *B*, medium viscosity 7%	Constant	33.3	83.3	91.7	91.7	100.0	100.0	25.0	41.7	91.7	91.7	91.7	100.0	0	8.3	83.3	100.0	100.0	100.0
	Alternating	0	0	0	0	8.3	25.0	25.0	33.3	33.3	33.3	75.0	75.0	0	0	0	0	0	16.7
7% acid to methyl red	Constant	25.0	58.3	83.3	91.7	100.0	100.0	8.3	8.3	66.7	83.3	83.3	100.0	0	8.3	58.3	83.3	83.3	100.0
	Alternating	0	0	0	8.3	33.3	75.0	8.3	33.3	33.3	33.3	75.0	75.0	0	8.3	8.3	8.3	41.7	50.0
7% acid to methyl red containing 2.8% dimethylol urea**	Alternating	8.3	8.3	8.3	16.7	33.3	75.0	0	33.3	91.7	100.0	100.0	100.0	0	8.3	8.3	8.3	16.7	33.3
14%	Constant	8.3	41.7	66.7	66.7	100.0	100.0	33.3	33.3	58.3	75.0	75.0	100.0	0	8.3	41.7	66.7	75.0	100.0
	Alternating	0	8.3	33.3	33.3	41.7	41.7	25.0	50.0	83.3	83.3	83.3	83.3	0	0	16.7	41.7	50.0	66.7
14% acid to methyl red	Constant	33.3	50.0	75.0	83.3	100.0	100.0	8.3	8.3	50.0	75.0	75.0	100.0	0	0	58.3	100.0	100.0	100.0
	Alternating	0	0	33.3	41.7	58.3	75.0	33.3	66.7	66.7	83.3	83.3	83.3	0	0	0	33.3	50.0	58.3
14% acid to methyl red containing 2.8% dimethylol urea**	Alternating	0	0	0	0	0	18.2	0	0	90.1	100.0	100.0	100.0	0	0	0	0	0	9.1

* *One dozen eggs at each condition of every treatment.*

** *Dimethylol urea added in the ratio of 4 parts for every 10 parts of polyvinyl alcohol.*

*** *Constant at 21° C. and 90% relative humidity.*

† *Alternated for three-day periods between 21°C. at 90% relative humidity and 4.4° C.*

alternating temperatures are presented in Table V. It will be seen from the percentage of eggs showing interior mould or rot that the storage life of treated eggs held at constant temperature was from two to four weeks longer than the control. A statistical analysis showed that these differences were significant. On the other hand when subjected to alternating temperature conditions the treated eggs were only slightly better in this respect than the controls with the exception of those receiving treatments containing dimethylol urea in all of which the storage life was extended four weeks, an improvement that was significant statistically. Although the external moulds on a number of eggs from both the constant and alternated temperature groups were less than on the control, no definite trend is indicated. The results of those treatments containing dimethylol urea were better than the control in the fifth and sixth weeks of storage but worse in the ninth and tenth. The polyvinyl alcohol coating possessed one disadvantage in that it gave mould growths a glossy sheen which made them more conspicuous than those on untreated eggs. It will be seen that formation of a large air cell was delayed in all the treated eggs and that within any one treatment the group subjected to alternating storage temperatures had the smaller air cells. The most usable concentration seemed to be 7% of the medium viscosity, type *B* polyvinyl alcohol.

Since mould and bacterial growth is slow at 4.4° C. and eggs subjected to alternating temperatures were actually held for only half the storage period at the lower temperature, the results at the end of a 10-week storage period of alternating temperatures may be compared with those obtained for eggs stored at constant temperature for five weeks. When this is done it will be seen that four of the alternated groups had greater interior spoilage and that all had more external mould and larger air cells. Such differences were statistically significant with the exception of air cell size in the treatment with 14% polyvinyl alcohol containing 5.6% dimethylol urea.

The observations and measurements presented in Table VI show the effect of a variety of treatments on the storage life and quality of eggs. From an examination of the data the treatments may be grouped according to their effectiveness in preserving eggs.

Excellent results were obtained by six treatments which, with one exception, consisted of dipping in 7% polyvinyl alcohol containing 2.8% dimethylol urea. These were as follows: dipped twice (No. 12), dipped first in commercial hydrogen peroxide (No. 17), dipped first in a saturated aqueous solution of dimethylol urea (No. 18), carbon dioxide conditioned before dipping (No. 19), carbon dioxide conditioned and dipped twice (No. 20), and carbon dioxide conditioned followed by dipping in warm melted vaseline instead of polyvinyl alcohol (No. 21). Although the vaseline dip proved to be the most effective sealing agent as judged by loss in weight, pH, white thickness, and yolk index measurements, it did not inhibit the growth of exterior mould. It is to be noted that the differences between these treatments and the control are for the most part highly significant statistically.

TABLE VI

EFFECT OF VARIOUS PRESERVATIVE TREATMENTS ON STORAGE LIFE AND QUALITY OF EGGS HELD AT 21° C. AND 90% RELATIVE HUMIDITY**

No.	Treatment	Percentage showing visible defects															Quality measurements at end of sixth week (Table of means)					
		Large air cell			Stuck yolk			Exterior mould			Interior mould			Interior rot			Loss in wt. per egg, gm.	Egg white			Yolk Index	
		Wk.			Wk.			Wk.			Wk.			Wk.				No. of eggs measured	pH	Thickness (height), in.	No. of eggs measured	Index
		4	5	6	4	5	6	4	5	6	4	5	6	4	5	6						
1	Control	13.3	20.0	20.0	13.3	40.0	53.3	40.0	40.0	46.7	13.3	53.3	53.3	0	40.0	53.3	2.2	7	9.21	0.037	4	0.233
2	Oiled and packed in sealed moisture resistant bags	0	0	0	0	0	6.7	0	20.0	20.0	0	0	0	0	0	0	1.1	15	8.89	0.056	12	0.293
3	Dip *A**	6.7	6.7	6.7	40.0	40.0	53.3	40.0	40.0	46.7	6.7	26.7	40.0	13.3	20.0	40.0	1.7	9	9.21	0.056	5	0.222
4	Commercial hydrogen peroxide, 3%	0	6.7	13.3	0	6.7	40.0	0	0	0	0	6.7	6.7	0	6.7	20.0	2.0	12	9.20	0.036	7	0.263
5	Saturated aqueous solution of dimethylol urea	0	6.7	13.3	0	0	0	0	0	0	0	0	0	0	0	6.7	2.5	14	9.10	0.083	10	0.409
6	Saturated aqueous solution of borax	6.7	13.3	13.3	0	6.7	6.7	0	0	0	0	0	0	0	6.7	6.7	1.4	14	9.22	0.037	8	0.257
7	Saturated aqueous solution of urea	0	0	0	6.7	13.3	26.7	0	0	6.7	0	0	0	6.7	6.7	6.7	1.5	14	9.14	0.053	7	0.262
8	Oiled with oil containing 0.03% IN-3102	0	0	0	73.3	86.7	100.0	100.0	100.0	100.0	40.0	73.3	80.0	40.0	66.7	93.3	0.9	4	7.24	0.082	3	0.335
9	7% polyvinyl alcohol	13.3	20.0	20.0	13.3	26.7	60.0	46.7	66.7	80.0	0	13.3	26.7	0	13.3	46.7	1.7	8	8.82	0.069	7	0.288
10	4% polyvinyl alcohol containing 1.6% dimethylol urea	0	0	0	6.7	13.3	13.3	6.7	6.7	13.3	0	13.3	13.3	0	13.3	13.3	1.2	12	8.94	0.044	4	0.268
11	7% polyvinyl alcohol containing 2.8% dimethylol urea	0	0	0	0	0	13.3	0	0	20.0	0	0	13.3	0	0	6.7	1.3	13	8.46	0.047	10	0.287
12	Dipped twice in 7% polyvinyl alcohol containing 2.8% dimethylol urea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.4	15	8.63	0.072	11	0.304
13	7% polyvinyl alcohol containing 5% urea	0	0	0	0	0	6.7	0	0	0	0	0	0	0	0	0	1.6	15	8.78	0.059	9	0.289
14	7% polyvinyl alcohol made alkaline with sodium hydroxide	0	0	0	6.7	13.3	60.0	26.7	26.7	33.3	6.7	13.3	33.3	0	6.7	26.7	1.3	11	8.74	0.055	7	0.296

* *Dip A, commercial solution for egg dipping believed to be oil or wax dissolved in a volatile solvent.*

** *Fifteen eggs used for each treatment and for each initial measurement.*

NOTE:—*Initial measurements were as follows. pH: normal = 8.83; CO_2 conditioned = 6.54. White thickness: normal = 0.134 CO_2 conditioned = 0.187. Yolk index: normal = 0.388; CO_2 conditioned = 0.410.*

TABLE VI—*Concluded*

EFFECT OF VARIOUS PRESERVATIVE TREATMENTS ON STORAGE LIFE AND QUALITY OF EGGS HELD AT 21° C. AND 90% RELATIVE HUMIDITY**—*Concluded*

No.	Treatment	Percentage showing visible defects															Quality measurements at end of sixth week (Table of means)					
		Large air cell			Stuck yolk			Exterior mould			Interior mould			Interior rot			Loss in wt. per egg, gm.	Egg white			Yolk Index	
		Wk.			Wk.			Wk.			Wk.			Wk.				No. of eggs measured	pH	Thickness (height), in.	No. of eggs measured	Index
		4	5	6	4	5	6	4	5	6	4	5	6	4	5	6						
15	7% polyvinyl alcohol containing 2.8% dimethylol urea and packed in Keyes trays treated with IN-3102	6.7	6.7	13.3	0	6.7	20.0	0	6.7	6.7	0	0	0	0	0	6.7	0.9	15	8.13	0.049	10	0.306
16	7% polyvinyl alcohol containing 2.8% dimethylol urea followed by formaldehyde dip	0	0	0	0	6.7	6.7	0	0	0	0	0	0	0	0	0	1.3	14	8.74	0.060	12	0.297
17	Commercial hydrogen peroxide followed by 7% polyvinyl alcohol containing 2.8% dimethylol urea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.2	14	8.76	0.048	8	0.305
18	Saturated aqueous solution dimethylol urea followed by 7% polyvinyl alcohol containing 2.8% dimethylol urea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.2	14	8.48	0.079	11	0.341
19	CO_2 conditioned followed by 7% polyvinyl alcohol containing 2.8% dimethylol urea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.4	14	8.26	0.135	12	0.361
20	CO_2 conditioned dipped twice in 7% polyvinyl alcohol containing 2.8% dimethylol urea	0	0	0	0	0	0	0	0	0	0	0	6.7	0	0	0	1.5	15	7.88	0.176	12	0.397
21	CO_2 conditioned followed by vaseline treatment	0	0	0	0	0	0	6.7	33.3	33.3	0	0	0	0	0	0	0.1	15	7.47	0.193	11	0.434
22	CO_2 conditioned followed by oiling	0	0	0	6.7	6.7	26.7	53.3	66.7	73.3	0	6.7	26.7	0	20.0	33.3	1.5	11	8.95	0.158	9	0.350
Maximum difference necessary to attain statistical significance (with the exception of treatment No. 8)																	0.51		0.31	0.332		0.050

* *Dip A, commercial solution for egg dipping believed to be oil or wax dissolved in a volatile solvent.*

** *Fifteen eggs used for each treatment and for each initial measurement.*

NOTE:—*Initial measurements were as follows. pH: normal = 8.83;* CO_2 *conditioned = 6.54. White thickness: normal = 0.134;* CO_2 *conditioned = 0.187. Yolk index: normal = 0.388;* CO_2 *conditioned = 0.410.*

Seven treatments may be considered as giving good results. Variations in these, however, were more marked than in the first group, the growth of micro-organisms being controlled without preserving other attributes of quality and vice versa. Nevertheless all the measurements with only a few minor exceptions show statistically significant differences from the control. These treatments were as follows: dipped in oil and packed in sealed moisture resistant bags (No. 2), dipped in a saturated aqueous solution of dimethylol urea (No. 5), dipped in a 7% polyvinyl alcohol solution containing 5% urea (No. 13) and the following dips in polyvinyl alcohol, 4% containing 1.6% dimethylol urea (No. 10), 7% containing 2.8% dimethylol urea (No. 11), 7% containing 2.8% dimethylol urea packed in Keyes trays impregnated with IN-3102 (No. 15), and 7% containing 2.8% dimethylol urea followed by a formaldehyde dip (No. 16).

A third group giving fair results included dips in a saturated aqueous solution of borax (No. 6), and of urea (No. 7), 7% polyvinyl alcohol made alkaline with sodium hydroxide (No. 14), and the carbon dioxide conditioned eggs dipped in oil (No. 22). The first two treatments controlled the growth of organisms but had little effect on the preservation of white thickness and yolk index while the last two maintained these qualities with only slight effect on the growth of micro-organisms.

Three treatments were little better than the control when all factors were considered. Although hydrogen peroxide (No. 4) reduced the growth of organisms it did little to preserve white thickness or yolk index. Dipping in 7% polyvinyl alcohol alone (No. 9) helped to retain normal white and yolk conditions but did not hinder mould or bacterial growth. Dip *A*, a commercial solution for egg dipping believed to be an oil or wax dissolved in a volatile solvent (No. 3), showed only slight improvement over the control.

The eggs dipped in oil combined with a low concentration (0.03%) of IN-3102 (No. 8) were the only ones whose condition was worse than the control. Some of the measurements on this treatment may be misleading since only a few eggs were left for measurement and these were on the verge of spoiling. The pH of a newly laid egg is of the order of 7.6. With the loss of carbon dioxide the pH rises and at ordinary storage temperatures reaches 9.4 in about one week. When spoilage occurs the pH drops again (1). In this treatment the eggs were sufficiently spoiled that the pH had dropped.

An analysis of covariance of the data provided by pH, white thickness, and yolk index measurements showed that yolk index and white thickness were positively correlated ($r = .76$), pH, and white thickness negatively correlated ($r = -.58$), and similarly pH and yolk index ($r = -.55$). All of these coefficients were statistically significant. In addition, statistical treatment of the results showed that the thinning of the thick white was not directly responsible for such a defect as stuck yolk within the limits of these tests. A similar examination showed, as might be expected, that the size of air cell was correlated with the weight loss. The mean weight loss in eggs classified as having large air cells was 2.80 gm., and in those with small air cells 1.34 gm.

Conclusions

The most satisfactory treatment for egg preservation would appear to be the one that effectively disinfects a newly laid egg and at the same time seals it against future contamination and loss of moisture and carbon dioxide.

None of the sealing agents (polyvinyl alcohol, oil, Dip *A*, vaseline) when used alone sealed the egg perfectly and none exhibited bacterial or mould growth inhibiting properties; in fact they all appeared to enhance the growth of surface mould. Vaseline was the best sealing agent. Double dipping in polyvinyl alcohol containing 2.8% dimethylol urea and packing oil dipped eggs in moisture resistant bags gave good results in retaining quality in all the attributes measured. Exposure in an atmosphere of carbon dioxide before dipping had a marked effect on the preservation of white thickness and yolk index.

Of the several disinfectants used (hydrogen peroxide, dimethylol urea, borax, urea, IN-3102), it has been shown that dimethylol urea even in small concentrations inhibits the growth of moulds. It also combines readily with polyvinyl alcohol thereby increasing resistance to the passage of water vapour and, since an excess was used, enhancing the mould growth inhibiting properties of the treatment. The fact that eggs immersed in oil containing IN-3102 were in a poorer condition than the control in many respects is no doubt explained by the indication that small amounts of the chemical stimulated mould growth. Although relatively high concentrations of IN-3102 vapour inhibited mould growth its use commercially is precluded by the unpleasant taint that it imparts to the egg. In flavour tests it was possible to detect its presence even when very small amounts were used and for this reason alone it would have to be rejected as a practical treatment. It is evident also that none of the disinfectants contributed anything other than hindering the growth of micro-organisms although dimethylol urea appeared to strengthen the vitelline membrane and to a lesser degree prevented the thinning of the thick white.

It is also evident that condensate formed when chilled eggs are brought in contact with warm, moist air is an important factor in the growth of exterior mould and that temperature changes also tend to increase the incidence of interior mould growth and spot rots.

Treatments that do not require the eggs to be dipped would probably be the easiest and most economical to apply commercially. This type, however, proved to be the least effective of those tested. Since oil dipping of eggs has been applied commercially, all treatments involving a single dip would appear to be practicable, provided the substance used can be applied as easily and at no greater cost than that for oil. The tested treatments of this type were generally of intermediate effectiveness although some of them were of little value. The most effective treatments were those involving either two dips or other two-stage treatments. Their commercial practicability cannot be assessed from these experiments since equipment and procedures for large scale operations have not been developed.

Acknowledgments

Grateful acknowledgment is due to Dr. W. H. Cook for many helpful suggestions, to Dr. J. W. Hopkins, Statistician, and to Messrs. M. W. Thistle, H. Tessier, E. C. Smith, F. H. Smith, D. A. Wright, M. L. McMahon, and L. Moore for assistance in making observations and measurements.

References

1. HAINES, R. B. Dept. Sci. Ind. Research (Brit.), Food Invest. Board, Special Rept. 47. 1939.
2. HAINES, R. B. and MORAN, T. J. Hyg. 40(4) : 453-461. 1940.
3. MALLMAN, W. L. and MICHAEL, C. E. Michigan Agr. Expt. Sta. Tech. Bull. 174. 1940.
4. ROSSER, F. T. Unpublished thesis, Univ. Western Ontario. 1936.

MARINE MACROPLANKTON FROM THE CANADIAN EASTERN ARCTIC

II. MEDUSAE, SIPHONOPHORA, CTENOPHORA, PTEROPODA, AND CHAETOGNATHA[1]

By M. J. Dunbar[2]

Abstract

Thirteen species of medusae, one siphonophoran, two ctenophores, two pteropod molluscs, and two chaetognaths are recorded from coastal waters of the Canadian eastern Arctic, many of them for the first time. All except one are known to be arctic or arctic–boreal species.

Hybocodon prolifer L. Agassiz has not hitherto been recorded from Arctic water. Specimens answering to this species were found by the author in large numbers at Lake Harbour, on Hudson Strait. The determination of the medusa is possibly not satisfactory, and cannot be considered certain until the hydroid is found. No species of *Hybocodon* has been recorded from Greenland water, however, and hence this discovery may be useful to distinguish Canadian polar water from water of the Greenland current.

Introduction

The material described in this paper was collected during the summers of 1939 and 1940, in the coastal water of Baffin Island and northern Labrador. Collecting stations were at Hebron, Port Burwell, Lake Harbour, Gabriel Strait, Frobisher Bay, Pangnirtung, Clyde River, Pond Inlet, Arctic Bay, and Fort Ross (13). The details of method, the hydrography of the area, a general introduction, and a map showing the collecting stations have been given in the first paper of this series (13). As in that paper, the convention of using parentheses around the names of authorities for species whose genus has been changed since the original naming of the species has been abandoned, following the practice of Osgood (42) and others.

I. MEDUSAE

ANTHOMEDUSAE

Family CODONIDAE

Sarsia tubulosa M. Sars

Syn. *Sarsia mirabilis* L. Agassiz

Taken at Hebron, Lake Harbour, Gabriel Strait, Frobisher Bay, Clyde River, and Arctic Bay. Common.

In considering *S. tubulosa* as synonymous with *S. mirabilis*, the lead of Kramp (31) has been followed. Kramp, working on the material of the "Ingolf" expedition, brought to an end a long-felt doubt as to the validity of Hartlaub's (22) many species of *Sarsia* by putting several together under the name *S. tubulosa*. Levinsen (37), in describing specimens from west Greenland, says: "When I refer these specimens . . . . . to this species [*mirabilis*] and not to *Sarsia tubulosa*, it is only because *Sarsia mirabilis* is found on the American coast. It is very difficult to distinguish sharply between the two species." Mayer (39) calls *mirabilis* "*Sarsia tubulosa*, var. *Sarsia mirabilis* L. Agassiz", and the differences that he quotes between the varieties are very slight. Hartlaub himself, who separates the two as species, is doubtful

[1] *Manuscript received September 5, 1941.*

Contribution from the Department of Zoology, McGill University, Montreal, Que., with financial aid from the National Research Council, Ottawa, Canada.

[2] *National Research Council Student.*

as to their validity: "Die Frage ob *Sarsia mirabilis* Ag. eine gute Art und nicht etwa identisch mit *S. tubulosa* Lesson . . . . . ist, kann einstweilen noch für unerledigt gelten. Die Medusen gleichen manchen der als *Sarsia tubulosa* aufgefassten, europäischen Quallen ganz ausserordentlich. Solange aber ein Vergleich authentischer Stücke nicht ausgefürht ist, lässt sich nichts bestimmtes sagen" (22). Such a comparison has now been made by Kramp (31): ". . . . Hartlaub has made an attempt to unravel the northern species, without, however, to attain a final result. I have never willingly believed in these many species, because my examination of material from Denmark, Norway, the Faeroe Islands, Iceland, and west Greenland demonstrated that each of the features which, according to Hartlaub, constitute the characteristics of the various species . . . . . might occur in material from different localities in every possible combination." The present material from the eastern Arctic shows the same variation in characters as does Kramp's material, for instance, the great variation in the development and shape of the apical canal.

S. tubulosa, then, in the broader sense, is known from the boreal and arctic coasts of Europe, the Atlantic coast of North America, the Pacific, Iceland, Greenland, and the Siberian Sea (22, 37, 51, 47, 20, 35, 28, 31, and 32). It has been recorded from Labrador (2), and from Newfoundland (19).

One abnormal specimen, with one radial canal branched into two, and with five tentacles, was taken at Lake Harbour.

Sarsia princeps Haeckel. Taken at Lake Harbour, Gabriel Strait, Frobisher Bay, and Fort Ross. Twenty specimens.

This species is easily distinguished by its large size, shape somewhat narrower than *S. tubulosa*, and above all by the club-shaped so-called apical canal, which widens out at the distal end. Kramp (31) points out that this canal is in reality no true canal, the greater part being solid. He adds that "the shape of the distal dilatation is subject to great variation." At Gabriel Strait, one abnormal specimen was taken in which the mouth was divided into three parts.

S. princeps has been found in the American Arctic at Collinson Point and Point Barrow, Alaska (5), and from Labrador and Newfoundland (2); it has also been recorded from Newfoundland by Pinhey (44), Frost (19), and Kramp (30).

Further distribution: *S. princeps* belongs to Arctic water. It has been recorded from Greenland, Spitsbergen, Barents Sea, and the arctic coasts of Europe.

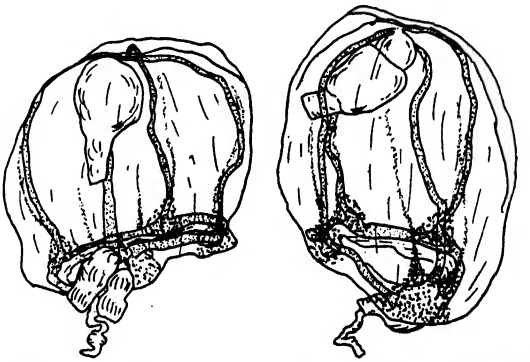


FIG. 1. *Diagram of two specimens of Hybocodon (?) prolifer L. Agassiz showing extremes of variation in width of the radial canal on the side bearing the tentacle.*

Hybocodon (?)prolifer L. Agassiz (Fig. 1). Taken in enormous numbers during July and August at Lake Harbour; about 5900 specimens in 1939, and 720 in 1940.

Hybocodon prolifer is very common in summer off the New England coast (20). It is known from Dutch Harbour on the Bering Sea (3), from Vancouver (16), southern Iceland, Norway, the British Isles (31), and from Newfoundland (19). The present specimens are identical with the description and figures of Hartlaub (22) and Kramp (31). The five rows of nematocysts are clearly visible, and their arrangement is typical. The length of the manubrium and the arrangement of the gonads also agree with previous descriptions; the length of some of the specimens is as much as 5 mm., as against 4 mm. quoted by Hartlaub. The radial canal on the side bearing the tentacle varies a little in width, the extremes being illustrated by the two specimens drawn in Fig. 1. It is never so broad as is described for

H. christinae Hartlaub, a form reported only from Bear Island and Bodö, in Norway. According to Bigelow (3), however, the medusae of *H. prolifer* and *H. christinae* closely resemble each other, although the hydroids are easily distinguishable. It seems best, therefore, to refer these specimens to *H. prolifer* provisionally, until the hydroids are found.

The record is interesting, and the distribution appears to be very local. On July 30 and 31, 1940, *Hybocodon* was taken at Lake Harbour; none was found at Gabriel Strait or Frobisher Bay between August 4 and 10, but on returning to Lake Harbour on August 14 large numbers appeared again in the plankton.

The record of *Hybocodon prolifer* from Newfoundland is published briefly in a report of the Newfoundland Fishery Research Commission. It is not qualified by date or locality, and after the species name, Frost has put "*prolifer* tentative" (19). It is possible, on the present record from Hudson Strait, that she found it in Arctic water, which is abundant at Newfoundland, particularly in the spring. From the distribution on the western side of the Atlantic this species would appear to belong to Lancaster Sound water and not to Greenland water. No species of *Hybocodon* has been recorded from the Greenland coasts, nor from Spitsbergen, and it is not known to be an arctic species at all. According to Kramp (31): "*Hybocodon prolifer* is a northern-boreal species, occurring all along the boreal coasts of Europe, but not penetrating into true arctic regions; it is, for example, neither found at the east coast of Iceland nor at the arctic coasts of Norway;" and Kramp and Damas (35) say: "Elle ne pénètre pas dans les régions strictement arctiques".

If these specimens should be confirmed as *H. prolifer*, on discovery of the hydroid, then the range of the species is considerably and remarkably extended. It is too early yet to put very much weight on the fact that it was taken at one station only; it is possible that the seasonal life of the medusa is short, and since the amount of tow-netting that has been done in the Canadian Arctic up to the present work is very small, the record must remain isolated pending further field work.

As to the determination of the specimens, they agree so closely with the existing descriptions of *H. prolifer* that there is little doubt of the determination being correct. In view of the present known distribution of the species, however, and since the hydroid form is not yet known from the same locality, it is necessary to treat the identification with reserve.

Family MARGELIDAE

Bougainvillia superciliaris L. Agassiz. Taken at Hebron, Lake Harbour, Gabriel Strait, and Frobisher Bay. Common.

The presence of a short but distinct peduncle, the inter-radial position of the gonads, and the development of the planulae while still attached to the gonads, fix these specimens as *B. superciliaris*. The number of tentacles in each bunch may be as high as 20. Hartlaub (23) says that the tentacle number per bunch in *superciliaris* is "11–15, seltener bis zu 22".

B. superciliaris has been recorded from Labrador, just south of Nain, by Bigelow (2), but from nowhere else in the American Arctic. It has been recorded from the Straits of Belle Isle (43), Newfoundland (19), Attu Island, in the north Pacific (3), west Greenland (28, 31, and 37), the east coast of North America (20, 4), Spitsbergen and Bear Island (1, 35), the North Sea, and the Norwegian coast (23).

According to Kramp (31) it is a native of Arctic water. Quoting from Kramp and Damas (35): "En allant du sud au nord, on passe du domaine de *Bougainvillia britannica* à celui de *B. principis* pour entrer dans celui de *B. superciliaris*, espèce circumpolaire."

Rathkea octopunctata M. Sars

Syn. *Rathkea blumenbachii* Rathke

Thirteen specimens, taken at Lake Harbour.

Recorded in Arctic America from Cooper Island, off Point Barrow (5). The synonymy of this species is rather complicated. Bigelow (2) records *Lizzia octopunctata* Forbes from Newfoundland, and considers it proved to be generically separated from *Rathkea octopunctata* Haeckel. Hartlaub (23) on the other hand, has decided that *L. octopunctata*, *R. octopunctata*, and *R. blumenbachii* are all synonymous. Mayer (39) puts *L. octopunctata* and *R. octopunctata* together, but separates *R. blumenbachii* from both of them. Kramp (31), who has made a study of much of the original material, has put all three together under the name *R. octopunctata* M. Sars, giving full reasons for retaining the name *R. octopunctata* rather than accepting *R. blumenbachii* as used by Hartlaub.

With the synonymy thus straightened by Kramp, this species, under the name *Lizzia octopunctata*, was recorded from Newfoundland, as mentioned above, by Bigelow. It is widely distributed in arctic and boreal seas, from Dutch Harbour (3) to the Barents Sea and the White Sea, and extending as far south as Bermuda and the Mediterranean (31). It is recorded also from the coast of British Columbia (51).

Family TIARIDAE

Halitholus pauper Hartlaub. Taken at Lake Harbour and Frobisher Bay. Very common during July and August.

Halitholus cirratus Hartlaub. Taken at Hebron, Lake Harbour, Gabriel Strait, Frobisher Bay, Arctic Bay, and Fort Ross. Common, but not caught in such large numbers as *H. pauper*.

The genus *Halitholus*, in the Arctic, is represented only by these two species, both first described by Hartlaub in 1914 (24). *H. cirratus* has been taken also in the Kattegat and Danzig Bay (24), and it has been recorded from the north coast of Alaska (5), and from Newfoundland waters (19). Fewkes' (15) *Tiara conifera*, from Grinnell Land, has been referred to *H. pauper* (24). These present records confirm the circumpolar distribution of the genus in the northern hemisphere.

Leuckartiara brevicornis (?) Murbach and Shearer. Taken at Lake Harbour.

The specimens are not fully mature, and identification is difficult. They have the horseshoe-shaped gonads typical of the genus *Leuckartiara*, and cannot therefore be referred to the next, and closely related genus, *Catablema*.

L. breviconis is known from Alaska, southwest Greenland, Iceland, southwest Norway, and north Scotland (31).

Catablema vesicaria A. Agassiz. Taken at Lake Harbour and Pond Inlet. Forty-six specimens.

Recorded from Labrador (2). *Catablema vesicaria* is an arctic and boreal species, known from West Greenland (31, 37), Iceland (31), Spitsbergen, Bear Island, Barents Sea (24), Bering Sea (3), Massachusetts Bay, and Woods Hole (20).

LEPTOMEDUSAE

Family MITROCOMIDAE

Tiaropsis multicirrata M. Sars

Syn. *Tiaropsis diademata* L. Agassiz

One specimen taken at Clyde River, September, 1940.

Recorded from the Bering Sea and North Pacific (3), Straits of Belle Isle and east Newfoundland (43, 44, 19), coasts of Europe from the English Channel to the Barents Sea and West Greenland (33), Massachusetts Bay, rare at Woods Hole (20).

TRACHYMEDUSAE

Family PTYCHOGASTRIIDAE

Ptychogastria polaris Allmann. One specimen from Lake Harbour, July, 1939.

A circumpolar Arctic form, known from the Bering Sea, Smith Sound, Davis Strait, Greenland coasts, Spitsbergen, Murman coast, Norway (9); Hebron and Cape Mugford, Labrador (2); also found further south in deep water; Nova Scotia (9); Winter Harbour (8). The most southerly record in Europe is from Hjörundfjord in west Norway (35).

Family TRACHYNEMIDAE

Aglantha digitale Müller. This very common northern and Arctic species was taken at Hebron, Burwell, all the Baffin Island stations, and Fort Ross, in large numbers.

NARCOMEDUSAE

Family AEGINIDAE

Aeginopsis laurenti Brandt. Taken at Hebron, Lake Harbour, Gabriel Strait, Frobisher Bay, Clyde River, Arctic Bay, and Fort Ross.

Recorded in the American Arctic from Collinson Point, Alaska (5), Labrador and Newfoundland (2), and the Bering Straits (7). Known also from Greenland (38 and 21), Norway (35), and many other stations in Arctic water. It is one of the most typical of Arctic medusae.

SCYPHOMEDUSAE

Small ephyrae of the Scyphomedusae, not identifiable further, were taken at Lake Harbour, Fort Ross, and Arctic Bay. Twenty-seven specimens.

II. SIPHONOPHORA

Family DIPHYIDAE

Diphyes arctica Chun. One specimen from Clyde River, September, 1940.

According to Kramp (27) *Diphyes arctica* is an Arctic water species. It was found by the Plankton Expedition of 1889 "in the boundary between the Gulf stream and the Irminger Sea", west of Greenland, in the North Sea, and at Bear Island and Spitsbergen (50, 10). In the Gulf of Maine it has been shown (6) to be an indicator, in the intermediate layers (below 50 metres), of mixed Arctic and Atlantic water.

III. CTENOPHORA

Family CYDIPPIDAE

Mertensia ovum Fabricius. Taken at Hebron, Lake Harbour, Gabriel Strait, Frobisher Bay, Pangnirtung, Arctic Bay, and Fort Ross. Common.

An Arctic species, almost certainly circumpolar (49, 2, 40).

Family BEROIDAE

Beroë cucumis Fabricius. Taken at Port Burwell, all the Baffin Island stations, and at Fort Ross. Very common.

An Arctic species, found at Labrador and Newfoundland (11, 2, 43, 44), Point Barrow (5), Davis Strait (14), and from Spitsbergen, north Pacific, Norwegian coasts, and the North Sea (49).

IV. PTEROPODA (Mollusca)

Family LIMACINIDAE

Limacina helicina Phipps. Taken at Hebron, Port Burwell, Lake Harbour, Fort Ross, Pond Inlet, Arctic Bay, Gabriel Strait, Frobisher Bay, and Clyde River; at Clyde River found also in the stomach of a ringed seal, *Phoca hispida* Schreber. Number of specimens, 2460.

An Arctic species "seldom found south of the 60th parallel" (35), but recorded from England and the coast of Provence by Odhner (41). This species has already been recorded from several stations in the eastern Arctic of Canada (26).

Family CLIONIDAE

Clione limacina Phipps. Taken at Hebron, Lake Harbour, Fort Ross, Pond Inlet, Arctic Bay, Pangnirtung, Gabriel Strait, and Clyde River. One hundred and sixty-seven specimens.

Found in all Arctic seas, off the coasts of Norway, Scotland, and the Atlantic coast of North America. It has already been recorded from many stations in the eastern Arctic (26).

V. CHAETOGNATHA

Sagitta elegans Verrill var. **arctica** Aurivillius. Taken at Hebron, Burwell, Fort Ross, and all the Baffin Island stations except Arctic Bay. Number of specimens, 4250.

Sagitta elegans arctica has been found in most Arctic waters, from Spitsbergen (46), west Greenland (29, 34, 12), east Greenland (32, 48), etc. The entry of cold water into the Gulf of St. Lawrence brings *arctica* with it (25). It has been recorded from the Firth of Forth and between the Faeroes and Shetlands (17, 18). It was found by the *Godthaab* expedition in Jones Sound, the mouth of Lancaster Sound, Cape Walsingham, and at the east end of Hudson Strait (34).

Eukrohnia hamata Möbius. Taken at Hebron, Lake Harbour, Frobisher Bay, and Clyde River. Twenty-two specimens.

Ritter-Zahony (45) gives the distribution of this species as "cosmopolitan, mesoplanktonic, in higher latitudes holoplanktonic. Bipolar". The *Godthaab* expedition found it at several stations on the west side of Baffin Bay, Davis Strait, and the Labrador Sea, as well as all up the west coast of Greenland (34).

Conclusion

As in the case of the amphipods of the present collection (13), the list published here is composed of species known to be arctic and arctic-boreal in distribution, with the one exception of *Hybocodon prolifer*. Four of the

species, namely *Ptychogastria polaris*, *Sarsia princeps*, *Aeginopsis laurenti*, and *Mertensia ovum*, are cited by Bigelow (5) as being typical Arctic species, and at all times and places sound indicators of Arctic water. The same is probably true of *Halitholus pauper*. Of the remainder, *Catablema vesicaria*, *Halitholus cirratus*, *Tiaropsis multicirrata*, *Bougainvillia superciliaris*, and *Diphyes arctica* have been found also in water of more temperate character, and others, notably *Rathkea octopunctata*, *Aglantha digitale*, *Leuckartiara brevicornis*, and *Sarsia tubulosa* are as much at home in northern Atlantic water as in Arctic water. The case of *Hybocodon prolifer* is exceptional, and must remain as a possibly most remarkable record until the hydroid is dredged. It has been pointed out (13) that the euphausiids *Thysanoessa inermis* Kröyer and *Thysanoessa raschii* M. Sars, and the mysid *Boreomysis nobilis* G. O. Sars may be useful in distinguishing Canadian polar water from Greenland water. The discovery of *Hybocodon* in the eastern Arctic has the same implications.

References

1. Aurivillius, C. W. S. Kgl. Svenska Vetenskapsakad. Handl., Ny följd, 32 (6) : 1-71. 1899.
2. Bigelow, H. B. Proc. U.S. Natl. Museum, 37 : 301-320. 1910.
3. Bigelow, H. B. Proc. U.S. Natl. Museum, 44 : 1–119. 1913.
4. Bigelow, H. B. Occasional Papers, Boston Soc. Nat. Hist. 7(12) : 1-37. 1914.
5. Bigelow, H. B. Can. Arctic Expedition, 1913-1918, 8 (H) : 1-22. 1920.
6. Bigelow, H. B. Bull. U.S. Bur. Fisheries, 40(2) : 1-509. 1926.
7. Brandt, J. F. Mém. Acad. St-Pétersbourg (Sér. 6), 4 : 237-411. 1838.
8. Broch, H. Rept. 2nd Norwegian Polar Expedition, 1898-1902, 2(12): 1-12. 1907.
9. Broch, H. Nord. Plankton, 6 : 481-540. 1929.
10. Chun, C. Ergeb. Plankton Expedition Humboldt-Stiftung, 2, K.b. : 1-126. 1897.
11. Chun, C. Ergeb. Plankton Expedition Humboldt-Stiftung, 2, K.a. : 1-32. 1898.
12. Dunbar, M. J. J. Anim. Ecol. 9(2) : 215-226. 1940.
13. Dunbar, M. J. Can. J. Research, D, 20(1) : 33-46. 1942.
14. Fabricius, O. Fauna Groenlandica. Hafniæ et Lipsiæ. 1780.
15. Fewkes, J. W. Rept. Proc. U.S. Expedition to Lady Franklin Bay, Grinnell Land, 2 : 39-45. 1888.
16. Fraser, C. M. Trans. Roy. Soc. Can. (Ser. 3) 8 (Sec. 4) : 99-216. 1914.
17. Fraser, J. H. J. conseil intern. exploration mer, 12 : 311-320. 1937.
18. Fraser, J. H. J. conseil intern. exploration mer, 14(1) : 25-34. 1939.
19. Frost, N. Newfoundland Ann. Rept. Fisheries Research Lab. 1936–1937 : 25-27. 1937.
20. Hargitt, C. W. Bull. U.S. Bureau Fisheries, 24 : 21-79. 1905.
21. Hartlaub, C. *In* Duc d'Orléans, Croisière océanographique accomplie à bord de la *Belgica* dans la mer du Grönland, 1905, (463-478). Charles Bulens, Bruxelles. 1907.
22. Hartlaub, C. Nord. Plankton, 6 : 1-136. 1907.
23. Hartlaub, C. Nord. Plankton, 6 : 137-236. 1911.
24. Hartlaub, C. Nord. Plankton, 6 : 237-364. 1914.
25. Huntsman, A. G. Can. Fisheries Expedition, 1914–1915 : 405-485. 1919.
26. Kerswill, C. J. J. Fisheries Research Board Can. 5(1) : 23-31. 1940.
27. Kramp, P. L. Bull. trimestriel conseil intern. exploration mer, 522-538. 1913.
28. Kramp, P. L. Vidensk. Medd. Naturh. Foren. 65 : 257-286. 1913.
29. Kramp, P. L. Vidensk. Medd. Naturh. Foren. 69 : 17-55. 1918.
30. Kramp, P. L. Rept. Sci. Results *Michael Sars* North Atlantic Deep Sea Expedition, 1910, 3(2) : (1-13). 1920.
31. Kramp, P. L. Danish Ingolf Expedition, 5(10) : 1-102. 1926.
32. Kramp, P. L. Medd. Grønland, 104(11) : 4-20. 1933.

33. KRAMP, P. L. Nord. Plankton, 6 : 541-602. 1933.
34. KRAMP, P. L. Medd. Grønland, 80(5) : 1-40. 1939.
35. KRAMP, P. L. and DAMAS, D. Vidensk. Medd. Naturh. Foren. 80 : 217-324. 1925.
36. LENZ, H. W. C. Nord. Plankton, 2 : 1-8. 1906.
37. LEVINSEN, G. M. R. Vidensk. Medd. Naturh. Foren. 44 : 143-212. 1893.
38. MAAS, O. Fauna Arctica, 4 : 479-526. 1906.
39. MAYER, A. G. Medusae of the world. Pub. 109. Carnegie Institution, Washington, D.C. 1910.
40. MORTENSEN, T. Rept. Sci. Results *Michael Sars* North Atlantic Deep Sea Expedition, 1910, 3(2) : (1-9). 1913.
41. ODHNER, N. H. Kgl. Svenska Vetenskapsakad. Handl., Ny följd, 54(1) : 1-274. 1915.
42. OSGOOD, W. H. Science, 89 : 9-11. 1939.
43. PINHEY, K. F. Contrib. Can. Biol. Fisheries (n.s.), 3(6) : 179-233. 1926.
44. PINHEY, K. F. Contrib. Can. Biol. Fisheries (n.s.), 3(13) : 331-346. 1927.
45. RITTER-ZAHONY, R. Deut. Südpolar Expedition, 1901–1903, 13 : 1-72. 1913.
46. STOTT, F. C. J. Anim. Ecol. 5(2) : 356-369. 1936.
47. THIEL, M. E. Fauna Arctica, 6(2) : 119-158. 1932.
48. USSING, H. H. Medd. Grønland, 100(7) : 1-108. 1938.
49. VANHÖFFEN, E. Nord. Plankton, 6 : 1-7. 1903.
50. VANHÖFFEN, E. Nord. Plankton, 6 : 9-39. 1906.
51. WAILES, G. H. Vancouver Museum Art Notes, 4 : 1-13. 1929.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. APRIL, 1942 NUMBER 4

HEPATOGENESIS OF THE ATLANTIC SALMON (*SALMO SALAR* L.)[1]

BY HELEN I. BATTLE[2]

Abstract

The anlage of the liver of the Atlantic salmon, *Salmo salar* L., arises during the eleventh week, 4.0 mm. embryo, as a proliferation of entodermal cells from the ventral wall of the anterior intestinal region. This anlage soon takes on a double appearance caudally. In embryos of 12 to 12½ weeks (4.7 to 5.0 mm.) it consists of right and left primordia with a common ventral opening into the intestine. By 13 weeks (5.4 mm. embryo) the anlage has curved toward the left, a position assumed through the counter-clockwise rotation of the intestine. The cells are becoming arranged in cords. A subsequent clockwise rotation of the intestine, apparent first in embryos of 14 weeks (6.5 mm.), draws the anlage to a dextro-ventral position. By 16 weeks (8.5 mm.) the ductus choledochus enters the right side of the intestine; the anlage itself passes posteriorly along the ventral surface of the intestine then abruptly forward in the form of a hook to the right of the intestine. The liver cells are arranged as branching trabeculae with some indication of vascularization. The liver anlage becomes rhomboidal and migrates to a position on the right of the rectilinear intestine, where it remains until some time after hatching. In the 25 mm. alevin (26 days after hatching) the intestine through a process of flexion commences to differentiate into cardiac and pyloric stomach, ascending and descending limbs of intestine. Simultaneously the papilla duodeni marking the opening of the ductus choledochus into the intestine is carried to a sinistrodorsal position opening into the ascending limb of the intestine at the level of the second row of pyloric caeca. Subsequent clockwise rotation of the ascending limb finally carries it to the sinistroventral margin. Yolk absorption now permits the liver to grow ventrad to the intestine, while the anterior progression of the intestinal loop is instrumental in its migration to the anterior end of the coelom. In the early parr the liver is somewhat triangular in shape with the apex posterior and in the mid-line. Through unequal growth the left median lobe in the late parr becomes much longer than the right, from which, however, it is never distinctly divided. It has now assumed the general appearance of the organ in the smolt and adult. Enlargement of the blind end of the ductus choledochus in the 21 week embryo (11.8 mm.) forms the gall bladder. Hepatic ducts are formed as branches of the primary ductus choledochus.

Introduction

The development of the liver of the teleostean fishes has been less extensively investigated than has that of other vertebrate types that serve more commonly as laboratory forms for embryological studies. This is particularly true for the Atlantic salmon (*Salmo salar* L.) since the literature reveals a single brief reference by Ziegler (22) to the initial appearance of this organ in the embryo. A survey of the development of the liver of this anadromous species thus offers many problems of interest, not merely during the embryonic phases, but also following the larval period when it undergoes an enlargement and a differential growth which accompanies the metamorphosis of other

[1] *Manuscript received December 12, 1941.*
Contribution from the Atlantic Biological Station, St. Andrews, N.B., and the Department of Zoology and Applied Biology, University of Western Ontario, London, Ont.

[2] *Associate Professor of Zoology.*

structures already observed as constituting the transition from parr to smolt. The present paper concerns gross morphological changes in the liver during development from the initial appearance of the anlage in the 4 mm. embryo to the adult. A later paper will deal with the simultaneous histogenesis.

Materials and Methods

The embryological materials used in the following study were obtained from a series of salmon embryos collected at the Birch Cove Hatchery of the Atlantic Biological Station, St. Andrews, N.B., during the winter of 1939–1940. The collection consisted of daily samples of stages from fertilization, on November 17, to embryos almost ready to hatch, on May 20. Fixation alternated daily between Bouin's fluid and Davidson's fluid, a formol–glycerine–alcohol–acetic mixture. The latter fixative prevented excessive hardening of the yolk material, and was consequently more satisfactory than Bouin's for material that required to be sectioned. The average temperature over the incubation period was 1.4° C., but, with the exception of four days, a minimum temperature of 0.5° to 1° C. was maintained from November 22 to April 23. For this reason the early developmental stages in this series are passed through at a considerably slower rate than are those in the adjacent St. John hatchery where the minimum temperature is of shorter duration.

Since development is a function of both temperature and time, Wallich (20) designated the age of fish embryos by what he termed "thermal units". One thermal unit (*t.u.*) corresponds to a temperature of 1° F. above 32° F. for a period of 24 hr., or is really a Fahrenheit-calorie–day. For example, a mean temperature of 37° F. for one day yields five thermal units. It is possible, when the incubation period for any series is known in days, as well as

TABLE I

AGE–LENGTH RELATIONSHIP IN DEVELOPING SALMON FROM BIRCH COVE HATCHERY, 1939–1940

Age, wk.	Length, mm.	Water temperature, °C.	Thermal units, Fahrenheit-calorie–day
(Nov. 17, 1939) 0	0.0	1.0 – 3.0	
1–10	0.0–3.6	0.5 – 3.0	
11	4.0	0.5 – 0.6	117.6
12	4.3	0.5 – 0.6	124.6
12 3/7	4.7	0.5 – 0.6	127.7
12 4/7	5.0	0.5 – 0.6	128.7
13	5.4	0.5 – 0.6	131.7
14	6.5	0.6 – 0.7	140.0
15	7.0	0.6 – 0.7	148.0
16	8.5	0.5 – 0.7	158.1
17	9.4	0.6 – 0.7	166.0
18	9.9	0.5 – 0.7	173.6
19	10.6	0.5 – 0.7	182.2
20	11.4	0.6 – 0.7	190.4
21	11.8	0.6 – 1.1	201.1
22	12.4	0.6 – 2.8	217.7
23	14.7	0.7 – 4.5	238.3
24	15.0	1.8 – 5.1	287.3
25	16.4	3.5 – 7.0	356.5
26	19.0	4.6 – 9.5	439.2

the mean daily temperature, to calculate the thermal units, thus expressing the age of an embryo as the sum of both time and temperature.

The age in weeks and in thermal units, the length (i.e., the average for eight individuals) and the temperature of the water are given in Table I for representative embryos studied.

Salmon alevin and fry were collected at two-day intervals at the St. John hatchery, New Brunswick, during May and June, 1939, and fixed in Bouin's fluid. The hatching took place approximately 26 weeks after fertilization. Their age and length relationships together with the average daily water temperature are given in Table II. The length measurements represent the average for 10 individuals.

TABLE II

AGE–LENGTH RELATIONSHIP IN SALMON ALEVIN AND FRY FROM ST. JOHN HATCHERY, 1939

Age (from hatching), days	Length, mm.	Water temperature, °C.	Age (from hatching), days	Length, mm.	Water temperature, °C.
(May 3, 1939) 0	18.5	4.7	(May 3, 1939) 24	25.0	15.0
2	20.0	4.7	26	25.0	14.4
4	20.5	5.3	28	25.0	14.2
6	21.0	5.3	30	25.5	14.2
8	21.5	8.1	32	25.5	13.9
10	22.0	9.5	34	26.0	14.7
12	22.5	10.3	36	26.0	15.3
14	23.0	10.3	38	26.0	16.2
16	23.5	10.9	40	26.5	17.8
18	24.0	12.6	42	26.5	17.5
20	24.5	12.8	44	27.0	15.3
22	24.5	14.5			

Formalin preserved specimens of late fry, early and late parr, smolt, and adults from the Margaree and Moser Rivers, Nova Scotia, and the St. John River, New Brunswick, as well as pregrilse taken from weirs on the Bay of Fundy were also examined.

Serial transverse sections of a carefully graded series of embryos, alevin, and fry were cut at 10 μ and stained with Ehrlich's haematoxylin and triosin. When the salient developmental stages were determined, one to two additional series were prepared in each instance for corroborative study. Because of the relatively large size of the embryos it is possible to observe the liver anlage even at very early stages by microdissection, using fine glass needles. Four to six specimens of a graded series corresponding to those used for the serial sections were dissected for each stage. The graphic method of reconstruction was used to determine the development of the biliary duct systems as well as the extent of the earliest anlagen.

As is well known the size of the embryo and alevin affords no absolute criterion of the state of the development of the fish. Thus in order to permit a careful comparison of the different specimens with one another and with the descriptions of other observers, in the following account the salient details of the formative stage of several structures besides that of the liver are given in each instance to present a general developmental picture.

Development

Initial Appearance of Anlage

The literature dealing with the organogenesis of the teleostean liver is concerned chiefly with its origin and very early differentiation. A number of investigators have observed the first anlage as a single solid ventral outgrowth of the intestine. Vogt (19, p. 155) briefly referred to the initial appearance of the liver in *Coregonus palaea* (Cuv.) as an accumulation of cells beneath the intestine at the level of the pectoral fins. Reighard (11) examined the wall-eyed pike, *Stizostedion vitreum* (Mitchill) and in one instance states that the liver is first apparent as a solid outgrowth from the ventral wall of the alimentary canal just posterior to the pharynx (p. 34); and in another, he states that the liver is formed as one or more hollow branching outgrowths from the tube (p. 61). Lereboullet (6) was the first to observe the liver in the trout (species?) embryo as a little cellular mass against the external wall of the stomach. Balfour (1, p. 78), Stöhr (17), and Laguesse (5) also using the trout (species?) noted that the earliest stage is a solid ventral diverticulum of entodermal cells from the duodenal region of the intestine. Göppert (4) considered that it had more the character of a sac-like protrusion in the salmon trout (*Trutta trutta* L.). Stricker (18) examining the latter species and Riggert (12), *Trutta fario* L. and *Trutta iridea* (Gibb.) corroborated the earlier observations of the solid character of the anlage. Price (10) recorded its appearance as a ventral outgrowth from the mid-gut in the whitefish, *Coregonus clupeaformis* (Mitchill).

In contrast to these observations Wilson (21) indicated the anlage as a solid outgrowth of the dorsal wall of the enteron just posterior to the pectoral fins in *Serranus atrarius* Jord. and Gibb. As it increases in size he observed its ventral growth between the ectoderm and the yolk sac. The possibility of a misinterpretation occurs here, since in a 100-hr. embryo this anlage is a dorsolateral thickening of the intestine, while by 112 hr., the liver lies directly on the opposite side of the intestine extending ventrally. Again, however, at 160 hr. a definite dorsal attachment to the intestine appears although the organ extends laterally to the ventral body wall.

A liver anlage arising from the lateral surface of the intestine has been observed in a number of teleosts. Chevey (3) found a ventrolateral location of the anlage in a nine day old embryo of the perch (*Perca fluviatilis* L.). He assumed, however, that it was originally ventral since the intestine had undergone some torsion. Ballantyne (2), in a general embryological survey of *Callichthys littoralis* Hancock, observed the first rudiment of the liver as a little pouch from the lateral wall of the alimentary canal just where it bulges to the left into the stomach rudiment. Pohlmann (9) states that the liver appears as a lateral thickening of the intestine in *Trichopodus trichopterus* (Pall). Through torsion of the intestine it comes to lie ventrally, pressing into the yolk. She found a similar condition in *Cichlasoma bimaculatum* L. and *Cichlasoma cutteri* Fowler, where the left lateral anlage is not a thickening

but an actual protrusion of the intestine. Through torsion of the latter the anlage becomes ventrolateral, the ductus choledochus migrating ventrally toward the right.

Embryo: 4.0 mm., 11 Weeks, 117.6 Thermal Units

No evidence of a liver anlage could be found in embryos from Birch Cove hatchery 10 weeks after fertilization. In the 4.0 mm. 11 week old embryo the anlage is readily identified as a proliferation of cells from the midventral wall of the gut (Figs. 1, 16).

The blastoderm in a 4 mm. embryo has overgrown slightly more than three-quarters of the yolk sphere, giving the appearance of a large yolk plug. Twenty-eight to 30 pairs of mesodermal somites are present. The brain region has expanded considerably over the posterior portion of the neural cord. Three primary divisions are evident, the forebrain, midbrain, and hindbrain. The optic anlage now a shallow cup lies lateral to the forebrain with which it is connected by the optic stalk. The lens placode is present opposite the mouth of the cup. The auditory placode has sunk deeply below the surface and has become almost completely cut off as a hollow sac, the auditory vesicle. No lumen is present in the pharyngeal or intestinal regions of the gut with the exception of short irregular distances which are patent in the intestine. The notochord is solid, its cells not yet appearing vacuolated. The appearance of the anlage at this stage is considerably in advance of that noted by several investigators for the trout. Laguesse (5) observed its appearance after closure of the blastopore and Stricker (18) noted it first in *Trutta trutta* L. when the posterior body segments were already extending freely beyond the yolk sphere and the head lifted free of the yolk.

The anlage commences anteriorly as a midventral rather blunt cellular projection of the pharynx at the mid-level of the auditory vesicle, a position that Stöhr (17) had previously noted for the trout. At this region it has an approximate depth of 0.06 mm. Passing posteriorly it extends for a distance of about 350 μ and becomes progressively deeper as the intestinal region is attained. In a typical transverse section of this region (Fig. 16) the intestinal wall is composed of columnar epithelial cells with oval or elliptical nuclei. Those composing the cellular mass of the anlage have somewhat more rounded nuclei, and are extremely active mitotically. The lumen of the intestine is a small spherical pore in some regions, a narrow vertical slit in others, and elsewhere not yet patent. The intestine and anlage at this level are 0.16 mm. in depth, 0.11 mm. of which may be considered to represent the primordial liver. A heavy layer of splanchnic mesoderm is apparent on either side of the anlage. Its cells become more flattened as they pass over the surface of the yolk. The ventral cells of the anlage rest directly on the yolk syncytium, in which very large chromatin laden nuclei are present.

Double Character of Anlage

The majority of investigators have observed a single liver anlage. Siwe (15) and Smallwood and Derrickson (16) believe that they can detect a double anlage. The latter condition regularly occurs in *Acanthias*, an elasmobranch, in which the liver arises as a pair of shallow lateral diverticula from the lateral walls of the ventral half of the gut (13). Siwe (15) described the development of the liver in *Trutta fario* L., and in *Cyprinus* and *Syngnathus* embryos. In the trout the anlage makes its first appearance 15 3/24 days after fertilization as a thickening of the intestinal wall directly cranial to the yolk sac. By 16 9/24 days it has developed into two primordia (ventral saccate evaginations) with a common midexcretory duct. For a relatively long time the two primordia are cranially separated and fused caudally in the median duct. Smallwood and Derrickson (16) found that the liver in *Cyprinus carpio* L. originates as two ventrolateral evaginations from the mid-gut at the time of hatching. Maxia (8), however, observed the origin of the liver in this species at a considerably earlier period prior to hatching, when it is first apparent as a solid mass of endodermal epithelium along the lateral intestinal wall.

Embryo: 4.7 mm., 12 3/7 Weeks, 127.7 Thermal Units

Embryos at this stage possess approximately 38 to 40 somites. The blastopore has closed, its position being indicated only by a slight furrow on the yolk sac in the caudal region. The embryo is prominently elevated above the yolk sphere and occupies about one-third of its circumference. The lens

---

Figs. 1 to 3 *are from graphic reconstructions;* Figs. 4 to 13 *from camera lucida drawings of dissections.*

Fig. 1. *Relationship of primary liver anlage to intestinal epithelium in the 4.0 mm. embryo. Ventral and right lateral aspects. Photomicrograph through the level X will be found in Plate I, Fig. 16.*

Fig. 2. *Relationship of primary liver anlage to intestinal epithelium in the 4.7 mm. embryo. Right lateral aspect. Photomicrograph through the levels X will be found in Plate I, Fig. 17.*

Fig. 3. *Relationship of the liver anlage and the intestinal epithelium in the 5.4 mm. embryo. Ventral and right lateral aspects. Photomicrographs through the levels X_1, X_2, and X_3, will be found in Plate I, Figs. 19, 20, 21.*

Fig. 4. *Ventral aspect, intestinal region of 6.5 mm. embryo showing first expansion of liver anlage toward the right.*

Fig. 5. *Ventral aspect, intestinal region of 8.5 mm. embryo showing migration of ductus choledochus, and whole liver anlage toward the right. Photomicrographs through the levels X_1 and X_2 will be found in Plate II, Figs. 22, 23.*

Fig. 6. *Ventral aspect, showing intestine and rhomboidal-shaped liver of 11.8 mm. embryo. Photomicrograph through the level X will be found in Plate II, Fig. 24.*

Fig. 7. *Ventral aspect of intestine and liver of 14.7 mm. embryo.*

Fig. 8. *Ventral and dorsal aspects of the intestine and liver of 22.5 mm. alevin (larva).*

Fig. 9. *Ventral aspect of intestine and liver of 25 mm. alevin (larva).*

Fig. 10. *Ventral aspect of digestive tract of 26 mm. alevin (larva).*

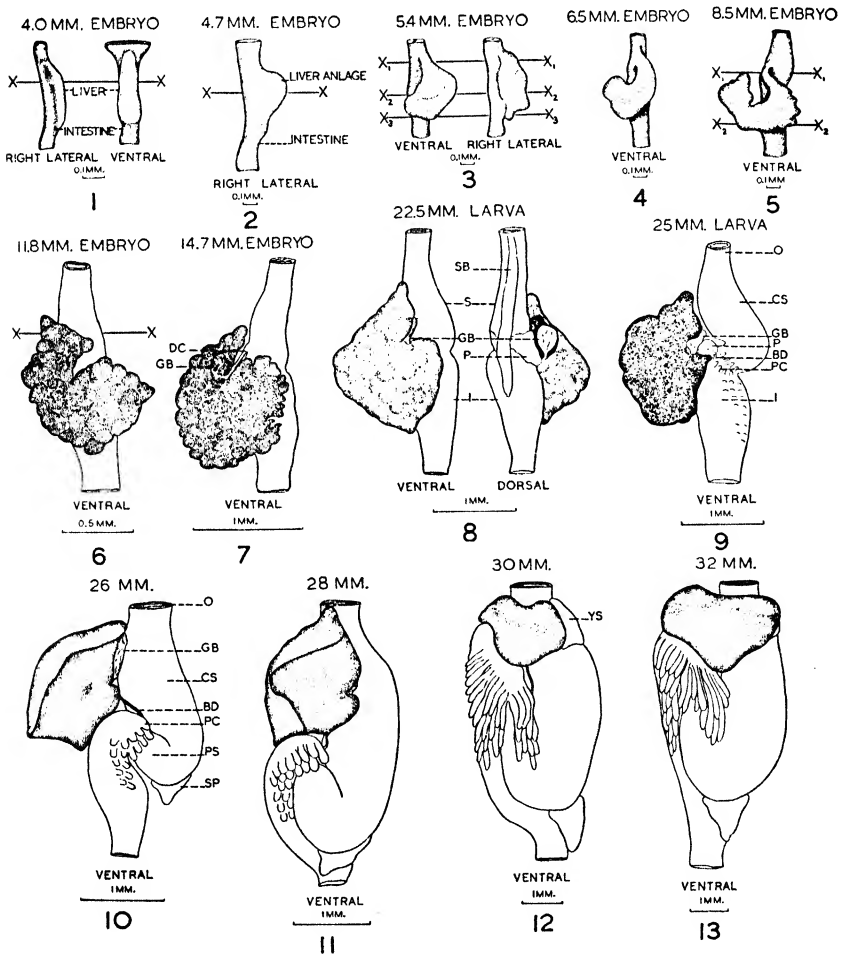
Fig. 11. *Ventral aspect of digestive tract of 28 mm. fry.*

Fig. 12. *Ventral aspect of digestive tract of 30 mm. fry.*

Fig. 13. *Ventral aspect of digestive tract of 32 mm. early parr.*

Abbreviations: C S = *cardiac stomach;* D C, B D = *ductus choledochus, common bile duct;* G B = *gall bladder;* I = *intestine;* O = *oesophagus;* P = *pancreas;* P C = *pyloric caeca;* P S = *pyloric stomach;* S = *stomach;* S P = *spleen;* S B = *swim bladder.*

has separated from the surface ectoderm and has migrated into the mouth of the optic cup. The auditory vesicle has enlarged and has sunk below the surface. The brain has increased in size. The cerebrum, pineal body, and infundibulum have arisen from the forebrain. The midbrain has formed the two optic lobes, while the hindbrain has differentiated into the metencephalon and myelencephalon. The dorsal fin fold extends from the head region posteriorly, surrounding the caudal region (knob) as the fore-runner of the caudal fin. Pectoral fins have appeared as lateral extensions of the body wall at the level of the first somites. The notochord is prominent with a few cells commencing to show the initiation of vacuolation as indicated by the peripheral migration of nuclei. Three pairs of dorsolateral branchial folds are formed on either side of the pharynx somewhat anterior and ventral to the ear. The



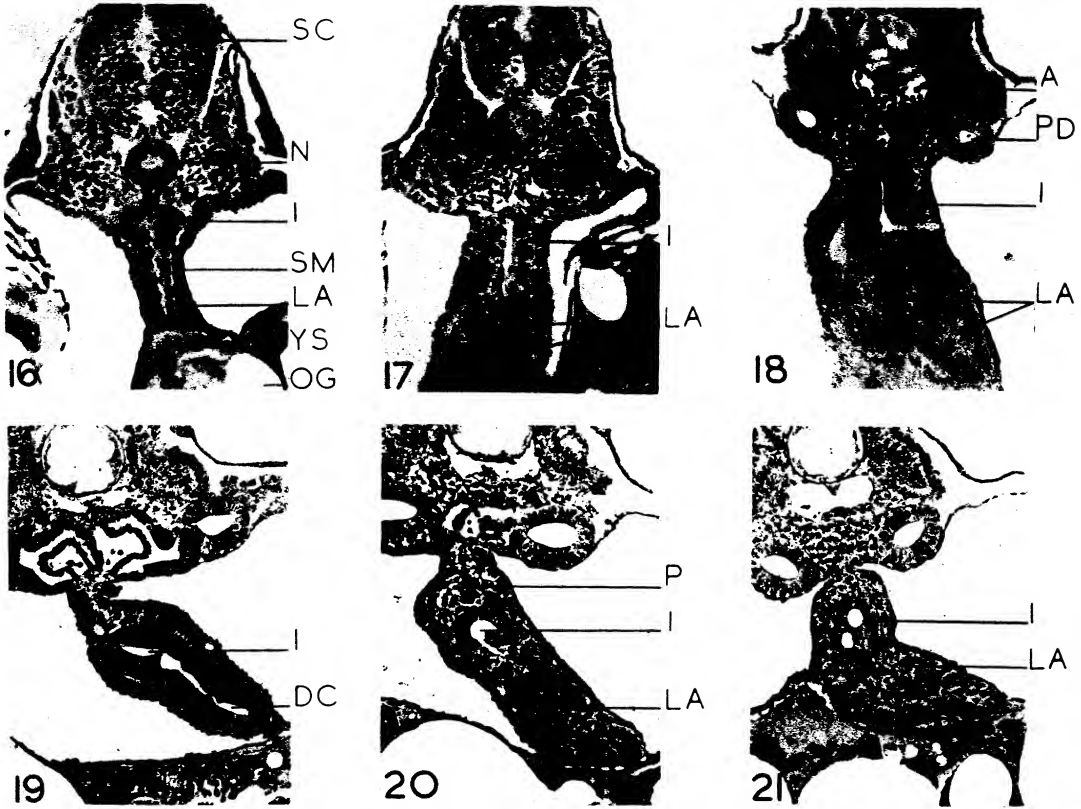
pharynx is still occluded, but posterior to it the gut now possesses a narrow dorsoventrally elongated lumen, which, however, is only intermittent along its course.

The anlage of the liver at this stage (Fig. 2) is about 0.4 mm. in length. As it projects ventrally it appears low cranially but rapidly becomes deeper. Its caudal margin declines gradually as it passes posteriorly and dorsally. In this respect it differs from *Salmo fario* L. (18), in which the caudal margin is directed very steeply dorsad. It is separated from the intestine by a very shallow longitudinal furrow. The anlage commences about 30 μ behind the auditory vesicle where it appears, as a somewhat blunt median ventral cellular proliferation about 0.08 mm. in depth. Passing posteriorly for about 60 μ it becomes a heavier cellular proliferation located ventral to the intestinal lumen and with a depth of 0.12 mm. It is composed of two or three layers of low columnar cells, sometimes irregularly arranged, in other instances radially. The nuclei are oval and active mitotically. Approximately 90 μ behind this (Fig. 17) the anlage takes on a double character with the left diverticulum appearing slightly anteriorly to the right. The maximal diagonal depth of either diverticulum from the intestinal lumen is about 0.11 mm. The cells at the base of each tend toward a radial arrangement but offer no distinct cytological differences from those forming the intestinal wall itself. They rest directly on the yolk syncytium. The splanchnic mesoderm covering the anlage laterally is of a low columnar type arranged in two or three layers. As it passes over the yolk surface the epithelium gradually becomes flattened and reduced to a single layer in thickness.

Embryo: 5.0 mm., 12 4/7 Weeks, 128.7 Thermal Units.

The anlage in this embryo is considerably advanced over the 4.7 mm. specimen. The pronephric duct has appeared and a vertically elongated intestinal lumen is present anteriorly along the course of the single anlage. Posteriorly the lumen appears triangular because of the entrance into it ventrolaterally of the ducts from the double anlage. For a distance of 0.15 mm. from its anterior extremity, the anlage has the character of a single cellular proliferation but posterior to this for about 0.26 mm. it takes on a double character (Fig. 18). The right diverticulum is somewhat smaller than the left having a maximal diagonal depth of 0.18 mm. from the base of the intestinal lumen, as compared to 0.23 mm. for the left. Consequent upon this, the whole structure is beginning to shift slightly toward the left. The epithelial cells composing the intestinal wall now appear to be pseudo-stratified columnar. Those of the solid anlage are characteristically low columnar and are rather irregularly arranged. The nuclei have become somewhat vesicular but are still actively mitotic.

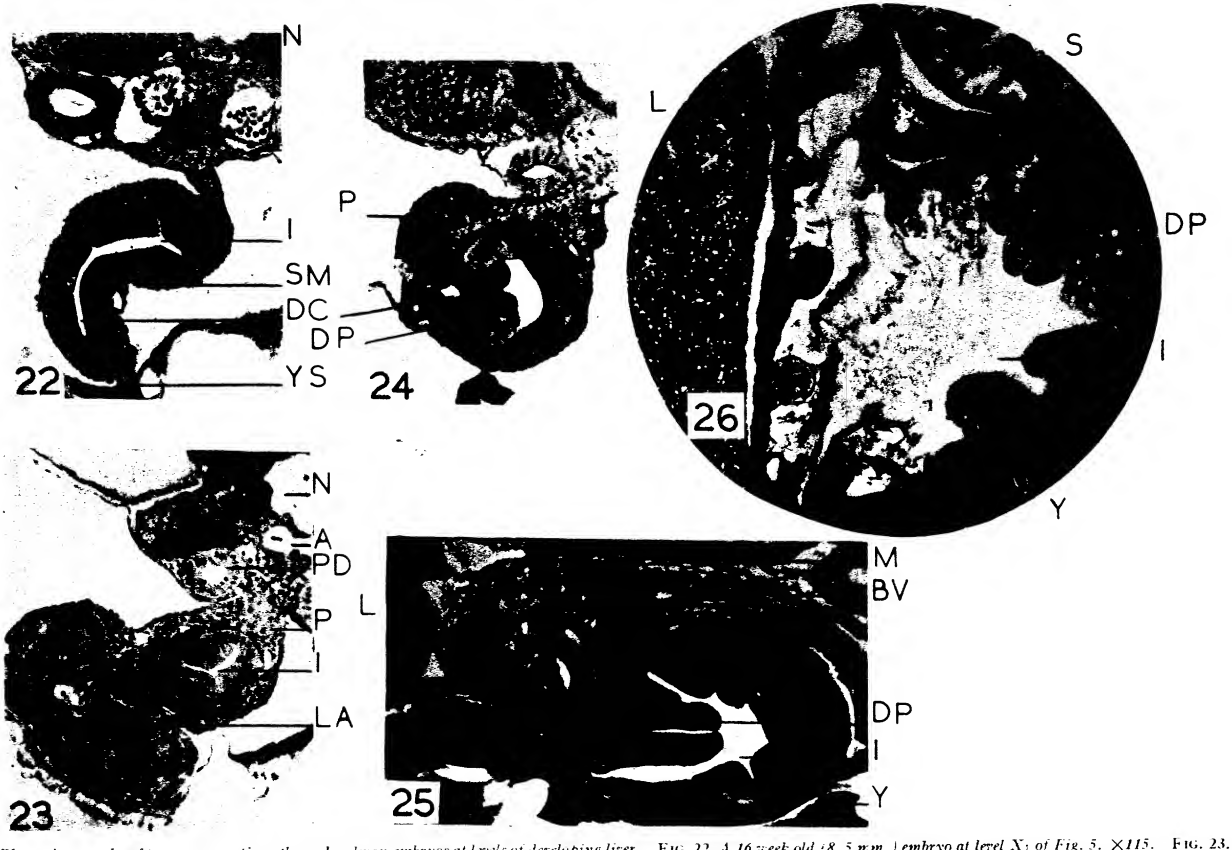
These conditions occurring in the salmon differ from those observed by Siwe (15) for *Trutta fario* L., where the two diverticula were observed to be separate cranially and fused caudally. In the salmon they appear single or fused cranially and are distinct from one another caudally.



Photomicrographs of transverse sections of salmon embryos illustrating the origin and differentiation of the liver. FIG. 16. *An 11 week old (4 mm.) embryo showing primary liver anlage at level X of Fig. 1. ×125.* FIG. 17. *A 12 3/7 week old (4.7 mm.) embryo at level X of Fig. 2. × 125.* FIG. 18. *A 12 4/7 week old (5.0 mm.) embryo through mid-level of liver anlage. ×115.* FIG. 19. *A 13 week old (5.4 mm.) embryo at level X1 of Fig. 3. ×115.* FIG. 20. *A 13 week old (5.4 mm.) embryo at level X2 of Fig. 3. ×115.* FIG. 21. *A 13 week old (5.4 mm.) embryo at level X3 of Fig. 3. ×115.*

Abbreviations: A = *aorta;* D C = *ductus choledochus;* I = *intestine;* L A = *liver anlage;* N = *notochord;* O G = *oil globule in yolk;* P = *dorsal pancreas;* P D = *pronephric duct;* S C = *spinal cord;* S M = *splanchnic mesoderm;* Y S = *yolk syncytium.*

PLATE I



Photomicrographs of transverse sections through salmon embryos at levels of developing liver. FIG. 22. *A 16 week old (8.5 mm.) embryo at level X1 of Fig. 5.* ×115. FIG. 23. *A 16 week old (8.5 mm.) embryo at level X2 of Fig. 5.* ×115. FIG. 24. *A 21 week old (11.8 mm.) embryo at level of papilla duodeni (X of Fig. 6).* ×90. FIG. 25. *Intestine of 22.5 mm. alevin (12 days after hatching) at level of papilla duodeni.* ×115. FIG. 26. *Intestine, stomach, and liver of 26 mm. alevin (36 days after hatching) at level of papilla duodeni.* ×115.

Abbreviations: A = *aorta;* B V = *blood vessel;* D C = *ductus choledochus;* D P = *papilla duodeni;* G B = *gall bladder;* I = *intestine;* L A = *liver;* N = *notochord;* M = *musculature;* P = *dorsal pancreas;* P D = *pronephric duct;* S = *stomach;* S M = *splanchnic mesoderm;* Y = *yolk;* Y S = *yolk syncytium.*

Torsion of the Anlage Toward the Left

Embryo: 5.4 mm., 13 Weeks, 131.7 Thermal Units

In an embryo at this stage of development approximately 50 somites are present. The head and tail regions have become raised off the yolk surface. The brain has increased greatly in volume, more especially by growth of the optic lobes and cerebellum. The optic cup now appears as a flattened sphere with a large cavity filled by the lens. Pectoral fins are well developed, and the median fin fold is continuous around the now laterally flattened tail, from the dorsal to the ventral median line. Vacuolation of the cells in the central region of the notochord is taking place. The lumen of the intestine appears about 0.3 mm. behind the ear. It is, however, quite irregular sometimes disappearing, at others becoming double.

The liver anlage (Fig. 3) once more appears as a single structure through union of the diverticula described in the preceding stages. It is still indistinguishably fused throughout its length of 0.43 mm. with the ventral wall of the intestine, but is no longer directed precisely ventrad, being folded over toward the left except at the posterior end. The original right lateral surface has become ventrolateral, the original left has become dorsolateral. This has been brought about by a counter-clockwise rotation of the intestine about its longitudinal axis, when viewed from the anterior end. Reference to transverse sections of the anlage at various levels (Figs. 19, 20, 21) will corroborate this. The anterior tip of the anlage appears 0.63 mm. posterior to the auditory vesicle approximately at the level of the anterior termination of the pronephric duct. The intestinal lumen (Fig. 19) is now no longer vertical, but through rotation of the intestinal tube has become horizontal. The ductus choledochus with a partially occluded lumen enters it on the left lateral border. The lining epithelial cells of both intestine and ductus are pseudostratified low columnar and are separated from the thickened splanchnic mesoderm by a very thin layer of connective tissue. The splanchnic mesoderm becomes flattened progressively from dorsal to ventral and that from either side meets to fuse ventral to the ductus choledochus, thus separating the latter from the yolk syncytium. About 0.1 mm. posterior to the previously described section, the intestinal lumen becomes irregular (Fig. 20). The splanchnic mesoderm is still flattened ventrally and serves temporarily as a thin separating layer between the hepatic cells and the yolk syncytium. The lumen disappears intermittently and may reappear in some irregular form such as the double character shown in Fig. 21, a section about 0.26 mm. from the anterior end of the anlage. Rotation of the intestine has not taken place to any extent at this level as indicated by the fact that the anlage still remains ventral, directly overlying the yolk syncytium. Had rotation taken place the splanchnic mesoderm could have grown medially from either side to meet ventral to the anlage. The cubical to low columnar hepatic cells are loosely arranged in the form of cords. There is some indication of loose connective tissue between them but no vascularization. The anlage at this level has a maximum diameter of 0.22 mm.

Although several investigators studied hepatogenesis in the trout, only Stricker (18) using *Trutta trutta* L. observed a temporary torsion of the early anlage toward the left, a condition resembling that just described for the salmon.

Migration of the Anlage Toward the Right

Göppert (4), Stöhr (17), and Stricker (18) showed that the original ventral liver anlage of the trout secondarily takes up a position to the right of the intestine. This was found to hold also for the salmon, except that the assumption of the dextral position was a very gradual process and not completely attained until shortly after hatching. Prior to that, most of the anlage lies on the right ventral surface of the intestine, the ductus choledochus entering its right lateral border.

Embryo: 6.5 mm., 14 Weeks, 140.0 Thermal Units

The embryo by continued growth has acquired the adult somite number of sixty. So far as external characteristics are concerned there is involved chiefly an increase in body size. The optic lobes are especially prominent but pigmentation is lacking. Through a secondary clockwise rotation or torsion of the long axis of the intestine when viewed from the anterior end, the anlage (Fig. 4) is gradually migrating from the left toward the right of the mid-line. This is accentuated as well by differential growth of the anlage itself, the posterior end now free of the intestine growing toward the right and anteriorly as a blunt projection. The maximum length of the anlage measuring from the entrance of the ductus choledochus posteriorly is 0.46 mm.

Embryo: 8.5 mm., 16 Weeks, 158.1 Thermal Units

Differentiation of the embryo is proceeding rapidly. The head is distinctly lifted off the yolk sac. The lens of the eye is commencing to develop crystalline fibres. A lumen has appeared in the pharynx, although the oesophagus is still occluded. The notochord is now composed entirely of vacuolated cells. The ventricular region of the heart has a conspicuous muscular development. The pectoral fins are large with a free thin terminal fringe.

The liver anlage (Fig. 5) has become hook-shaped, and has a maximal anterior–posterior length of 0.53 mm. The whole structure has migrated further toward the right but its left margin still extends over the corresponding margin of the intestine. The ductus choledochus enters the right lateral border of the intestine (Fig. 22). There is a cessation of rotation of the latter now until after hatching. The point of entrance of the ductus is 0.74 mm. from the posterior margin of the auditory vesicle and 0.11 mm. posterior to the anterior limit of the pronephric duct. The intestinal lumen is once more laterally flattened, although there is a tendency toward a horizontal shifting as indicated by its oblique position. The epithelial lining of the ductus is simple cubical or columnar, that of the intestine pseudostratified columnar. This type of epithelium is characteristic of the teleostean intestine (14, p. 174). The splanchnic mesoderm on either side of the intestine is heavy but becomes low and flattened ventrally as that from either side meets to form a ventral

mesentery between the ductus and the yolk. The epithelium of the intestine and ductus choledochus are covered externally by a thin connective tissue layer. The portion of the liver anlage lying immediately ventral to the intestine is still fused with the latter along the right ventrolateral surface, while that of the right anteriorly directed hook is free, a condition similar to that in the trout (18). Near the posterior end of the anlage (Fig. 23) the hepatic tissue measures about 0.4 mm. at its greatest diameter. The intestinal lumen is still somewhat oblique. The peritoneal epithelium has become flattened and scattered cells separate the ventral surface of the liver from the yolk. The hepatic cell cords are separated by connective tissue and numerous scattered vascular areas occur throughout the organ.

Subsequent Development of the Anlage

The liver remains in a right ventral position until shortly after hatching, but during this period is constantly shifting until it finally comes to lie entirely along the right margin of the intestine.

Embryo: 11.8 mm., 21 Weeks, 201.1 Thermal Units

In embryos of this stage the semicircular canals are commencing to develop in the ears. The oesophageal lumen is still occluded. Anteriorly the intestinal lumen is cylindrical, but posteriorly it usually becomes flattened dorsoventrally. The shape of the liver (Fig. 6) is not essentially different from that previously described, but it appears finely lobulated on the surface. It is somewhat rhomboidal in outline with one diagonal axis (0.80 mm. in length) passing parallel to the longitudinal axis of the intestine, the other transversely across the body at right angles. The cranial apex forms a hook on whose dorsal surface lies part of the pancreas and the ductus choledochus. The latter, arising from the liver, ascends rather abruptly and, passing transversely toward the left, discharges into the right lateral border of the intestine. At its point of entrance (Fig. 24) the intestinal wall is elevated to form the papilla duodeni which is at a level about 1 mm. posterior to the ear and projects 0.15 mm. into the lumen of the intestine. The pancreatic duct also empties into the papilla duodeni at a level slightly posterior to the opening of the ductus choledochus. On the dorsal surface of the liver the ductus choledochus is expanded into an elongated sac, the gall bladder. The intestine is lined with pseudostratified columnar epithelium with a cuticular border. It becomes simple columnar over the papilla duodeni and somewhat cubical in the ductus choledochus. The gall bladder is lined with cubical to low columnar epithelium. The cells of the liver are cubical with spherical nuclei and now appear to be arranged as branching trabeculae separated by large venous sinuses.

Embryo: 14.7 mm., 23 Weeks, 238.3 Thermal Units

Embryos at this stage possess melanic pigmentation in the choroid coat of the eye. Finely stellate melanophores are also appearing over the dorsal surface of the head and the trunk musculature. The pectoral fins are no

longer fleshy but thin and supported by rays. The gill slits are patent, but the oesophageal lumen is still occluded.

The liver (Fig. 7) is still somewhat rhomboidal, and covers the ventral surface of the intestine, but in most instances has a tendency to shift further toward the right. It is approximately 1.15 mm. in length. The intestine has acquired connective tissue and muscular coats about the epithelial lining, and a thin connective tissue coat also surrounds the epithelium of the gall bladder.

Embryo: 19 mm., 26 Weeks, 439.2 Thermal Units

Embryos at this stage of development have usually hatched, although not in this particular series. The body now forms a complete circle about the yolk sphere, the tail extending past the head sometimes as far as the margin of the operculum. Pelvic fins have appeared as fleshy buds at the posterior junction of the yolk sac with the ventral trunk musculature. The body axis lies with its right surface against the yolk, and the liver now occupies a position bordering on the yolk syncytium and almost entirely to the right of the digestive tract. The oesophagus is still occluded, and the anterior limit of the intestinal lumen is approximately the level of the anterior end of the pronephric duct. The right anterior tip of the liver appears 0.17 mm. in front of the level of the papilla duodeni which now projects about 0.18 mm. into the intestine. The papilla is covered by low columnar epithelium and possesses a connective tissue core surrounding the cubical to low columnar epithelium of the ductus choledochus. The liver has a maximum vertical depth of 0.9 mm. and a length of about 1.25 mm.

Alevin: 22.5 mm., St. John, 12 Days after Hatching

The alevin still has a heavy yolk sac. The eyes are deeply pigmented and scattered melanophores occur over the dorsal body surface. The dorsal and ventral fin folds are becoming differentiated. The gill slits are covered by the operculum. The digestive tract is still rectilinear (Fig. 8) and possesses a lumen except in the oesophageal regions. The liver has a sharply pointed right anterior projection, is broadest at mid-level and gradually tapers to its blunt posterior termination. It has a maximum length varying from 1.55 to 1.65 mm. and is situated on the right side of the intestine, curving slightly dorsal to it, and not infrequently may still project ventrally from the mid-level to the posterior end. The papilla duodeni (Fig. 25) enters the right side of the intestine about 0.4 mm. from the anterior tip, and now projects 0.2 mm. into the lumen. The liver at the level of the gall bladder and the first of the hepatic ducts, appears somewhat triangular in sectional area. Its dorsal surface is bounded by the body musculature, its left by the intestine, and the ventral by the yolk. Sinusoids occur between the trabeculae and larger veins are prominent about the gall bladder and duct.

Alevin: 25 mm., St. John, 24 Days after Hatching

At this stage the digestive tract is ceasing to be rectilinear (Fig. 9). In the adult salmon (Fig. 15) the oesophagus, cardiac stomach, pyloric stomach,

and ascending limb of the intestine (pyloric limb or duodenum) form an almost perfect *U*. From the pharynx, the oesophagus and cardiac stomach pass directly posterior for a distance equal to approximately one-half the length of the coelom. The tract then bends sharply on itself and passes directly anterior. The proximal half of this forward directed limb is the pyloric stomach, and the distal half, the ascending limb of the intestine, the pylorus marking the line of division between them. At the anterior end of the ascending limb of the intestine, another sharp flexure brings the small intestine directly posterior, that is, it forms the descending limb of the intestine which passes straight to the anus. Rotation at the pylorus has carried the descending limb of the intestine dorsolateral to the pyloric stomach and the ascending limb of the intestine. Pyloric caeca originate from the ascending limb of the intestine and from the region of flexure between it and the descending limb of the intestine. The initiation of the first or stomachal flexure in the digestive tract is apparent at the 25 mm. stage, somewhat anterior to the two primary rows of pyloric caeca anlagen. This flexion is the first indication of the division of the stomach into cardiac and pyloric limbs. The liver now lies wholly on the right side of the digestive tract and has a length of 1.6 to 1.7 mm. Its anterior extremity is sharply pointed. The left lateral margin appears somewhat irregular, the posterior is broad and blunt. Stricker (18) described in the larval stages of the trout, a somewhat rhomboidal-shaped liver with a right cranial hook comparable to that observed here in the salmon. He did not, however, carry development beyond the initial appearance of the first flexure in the digestive tract and through the subsequent migration of the papilla duodeni.

Alevin: 26 mm., St. John, 36 Days after Hatching

In this alevin the yolk sac has not noticeably decreased in extent except dorsolaterally, where its right lateral margin is now marked by an elongated ridge on the ventral surface of the liver (Fig. 10). Flexion of the digestive tract has increased and the ascending limb of the intestine has pushed forward against the right posterior surface of the liver. A transverse section at the level of the papilla duodeni (Fig. 26) passes directly through the intestinal flexure. The fact that the papilla duodeni has assumed a sinistrodorsal position is revealed. It is located now at the level of the union of the ascending and descending intestinal loops. At this time flexion of the intestinal tract is practically complete but torsion or rotation is just commencing. The displacement of the papilla duodeni through about 120° has occurred around the dorsal side of the intestine in a clockwise direction, if the tract be viewed from the anterior end. Simple flexion without torsion can account for this displacement.

Considerable histological differentiation is evident in the stomach and intestinal regions where the epithelial, connective tissue, and muscular layers typical of the adult have made their appearance. The liver cell trabeculae are more compactly arranged than in earlier stages.

Fry and Early Parr: 28, 30, 32 mm., St. John Hatchery, 1935

Further changes in the morphology of the liver occur simultaneously with yolk absorption and with the continued anterior growth of the ascending intestinal limb. The liver comes to occupy a position at the anterior end of the coelom just posterior to the septum transversum. In the 28 mm. fry (Fig. 11) the limit of the yolk sac is outlined on the lower surface of the liver. The posterior margin of the latter is gradually pushing toward the left and anteriorly immediately in front of the intestine. In addition to the original flexion, the gut has now undergone a torsion or rotation involving the pyloric stomach, the ascending and descending limbs of the intestine. As a result, the papilla duodeni has been rotated through 210° from its original position and now occupies a sinistroventral location on the ascending limb of the intestine immediately beyond the pylorus. The further displacement can only be attributed to about 90° of clockwise rotation of the tract when viewed from the anterior aspect.

At 30 mm. (Fig. 12) the yolk sac is absorbed with the exception of a triangular remnant adhering to the left lateral surface of the liver. The liver itself has assumed a somewhat triangular or cordate form, the former posterior margin now being directed to the left and posterior. The 32 mm. early parr (Fig. 13) possesses only a fragment of yolk at the left border of the liver. The latter is triangular in outline from the ventral view with the apex of the triangle directed toward the pylorus. The right lobe is growing posteriorly to cover the anterior part of the intestinal loop.

Liver–Yolk Relationship: St. John Hatchery Alevin, Fry, and Parr

The absorption of yolk is a major factor in the growth of the liver ventrally and toward the left from its early position to the right of the gut. Up to and including the 20 mm. alevin the liver lies directly on the dorsal surface of the yolk immediately to the right of the intestine. At 25 mm. a considerable quantity of yolk has been absorbed and the liver commences to grow ventrally from the right side toward the mid-line. The ventral growth continues and finally, disappearance of the yolk permits the liver to grow to the left and dorsally (31 mm.). It occupies most of the anterior end of the coelom about the stomach, the latter fitting into the gastric impression on its dorsal surface.

Development of the Biliary System

The relationship of the intestinal lumen to that of the biliary system has been illustrated by a series of graphic reconstructions of various developmental stages (Fig. 14, *a* to *i*). The intestinal lumen appears as a narrow irregular vertical slit at 3.8 mm. At 4.7 mm., when the anlage appears double in character, the lumen has become considerably deeper vertically. An embryo of 5.4 mm. possesses an irregular intestinal lumen which is joined anteriorly by the narrow ductus choledochus entering from the left side. The latter has an incomplete lumen which disappears as it reaches the anlage proper. By 6.5 mm. the anlage is shifting toward the right but the ductus still enters from the left. In the 8.5 mm. embryo through the clockwise rotation of the

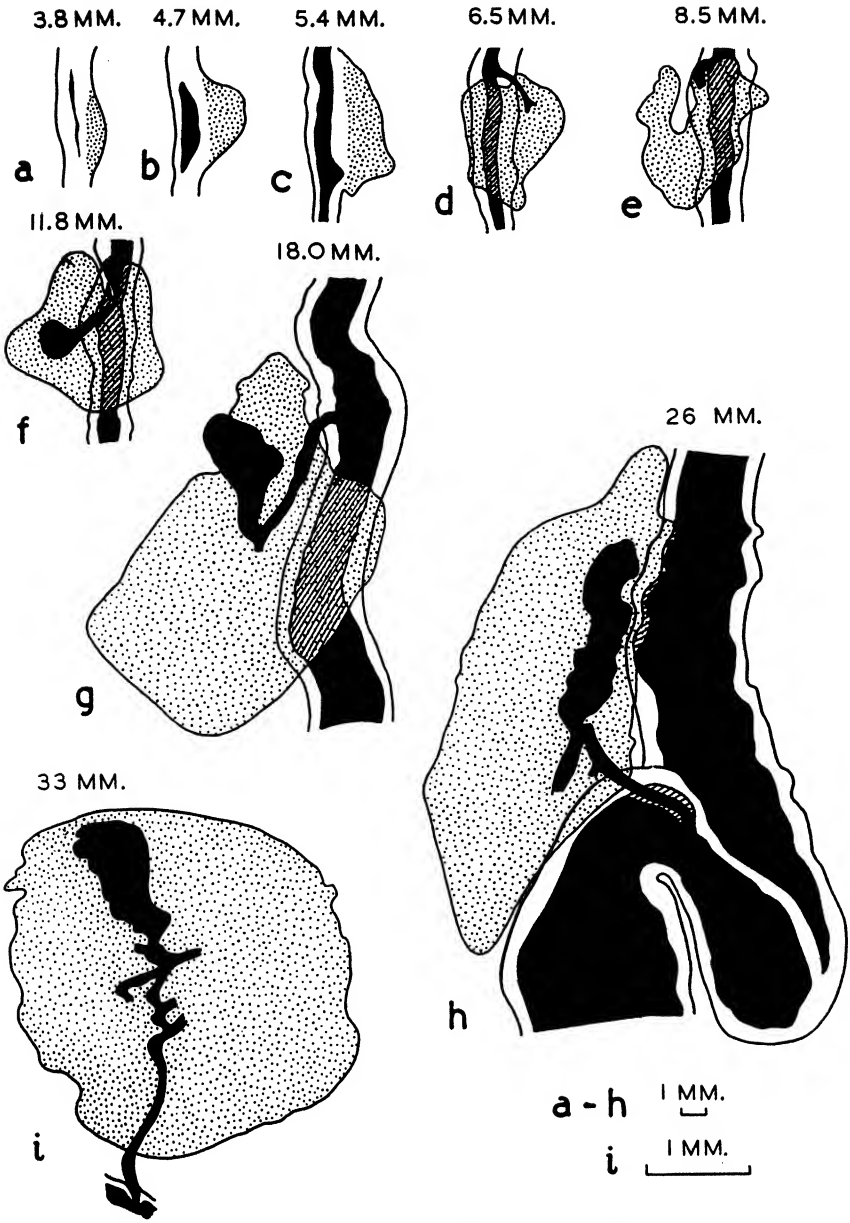


FIG. 14. *Graphic reconstruction of intestine (outline), liver (stippled), intestinal lumen and biliary system (solid black and shaded) in embryos, alevin, fry, and early parr at lengths of (a) 3.8 mm., (b) 4.7 mm., (c) 5.4 mm., (d) 6.5 mm., (e) 8.5 mm., (f) 11.8 nm., (g) 18.0 mm., (h) 26 mm., and (i) 33 mm.*

intestine, the ductus choledochus now joins the lumen of the latter from the right. The lumen of the ductus itself extends only from the intestine to the hepatic tissue. The first indication of the gall bladder is found in the 11.8 mm. embryo as an expansion of the posterior termination of the ductus choledochus on the dorsal surface of the liver. At 18 mm. the gall bladder has commenced to grow anteriorly thus forming an acute angle with the ductus. The first branch from the cystic duct is indicated passing into the liver parenchyma. Through flexion of the original rectilinear digestive tube, the liver is carried anteriorly in front of the intestinal loop (26 mm.). The ductus choledochus once more in linear relationship with the gall bladder enters the sinistrodorsal angle of the ascending limb of the intestine. At 33 mm. the liver, now occupying a position at the anterior end of the coelom, has on its dorsal surface anteriorly an elongated gall bladder, which drains into a curved cystic duct. The latter continues as the common bile duct, which receives several hepatic ducts from the liver substance. Torsion of the gut has caused its point of entrance to shift to the sinistroventral margin of the ascending limb of the intestine at the level of the second row of pyloric caeca.

Gross Morphology of the Liver

Parr and Smolt

After its migration to the anterior end of the coelom, the liver undergoes a gradual change in its general form (Fig. 15) through unequal growth toward the left. In the early parr it is somewhat triangular when viewed from the ventral surface, with the apex of the triangle directed caudad along the mid-ventral line (Fig. 15, *a* and *b*). Dorsally it is concave where it forms the gastric impression on the left, the intestinal impression on the right to conform with the contours of the cardiac stomach, and intestinal loop, respectively. Gradually the left half of the liver commences a posterior growth (Fig. 15, *c* to *f*). Prior to its transformation into a smolt, the liver of the late parr has already assumed the general proportions characteristic of the smolt (Fig. 15, *g* and *h*) and adult (Fig. 15*i*). This unequal growth of the liver may thus be considered as one of the changes accompanying the general metamorphosis of parr to smolt. The maximum anterior–posterior length of the liver in the parr is approximately one-tenth the body length. This proportion changes gradually until in the adult it is one-sixth.

FIG. 15. *Liver of representative parr and smolt from Nova Scotia and adults from St. John River, N.B.*

Parr: Ventral aspect, (a) 34 mm., Margaree River, August, 1935; (b) 40 mm., Moser River, August, 1939; (c) 54 mm., Moser River, August, 1939; (d) 63 mm., Moser River, August, 1939; (e) 158 mm., Margaree River, August, 1935; (f) 212 mm., Larry River, N.S., August, 1936.

Smolt: Ventral aspect, (g) 125 mm., Margaree River, June, 1938; (h) 220 mm., Lake Ainslee, August, 1935.

Adult: (i) Ventral aspect (92 cm. specimen) showing relationship of liver to adjacent viscera; (j) ventrolateral aspect (84 cm. specimen) showing posterior forking of left lobe and lappets; (k) dorsal, and (l) anterior aspect of same specimen as in (i).

Adult Salmon

The liver in the adult (Fig. 15, *i* to *l*) is a large yellowish- or greenish-gray to reddish-yellow gland situated at the anterior extremity of the body cavity. It is approximately one-sixth of the body length and imperfectly divided into

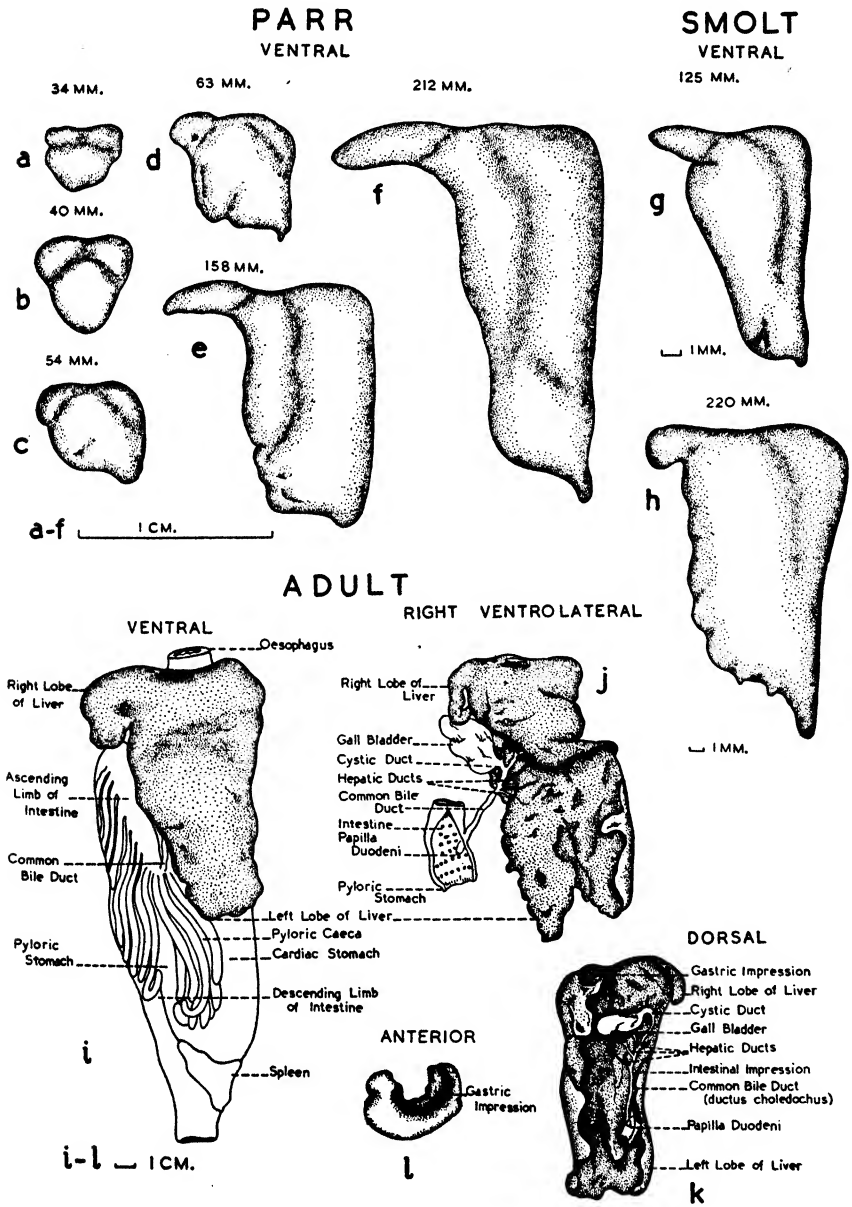


FIG. 15

a small right lobe and a larger left median lobe. The anterior and ventral surfaces are convex, while the dorsal or visceral surface fits the convexity of the stomach and the duodenum, providing the gastric and intestinal impressions. The organ is thick anteriorly but gradually becomes thin along the posterior and lateral margins which are composed of a number of dorsally flexed lappets similar to those described by Macallum (7) for *Ameiurus*. The right lobe is short, terminating immediately anterior to the intestinal flexure. The left lobe extends posteriorly over the stomach and the ascending limb of the intestine beyond the level of the entrance of the bile duct into the latter. The posterior margin of the lobe is not infrequently forked (Fig. 15*j*). The gall bladder is a somewhat conical or pear-shaped structure lodged in a fossa on the dorsal surface of the right lobe, with its blind end extending anteriorly and toward the left. Its long axis may extend transversely or diagonally from the gastric impression toward the right lobe where it passes into an arch-like cystic duct. The latter receives two or more ducts from the liver substance (hepatocystic ducts of Macallum (7)) and, in its posterior course, four or five hepatic ducts, whence it becomes the ductus choledochus (common bile duct). It enters the left ventral surface of the ascending limb of the intestine (duodenum) at the level of the second row of pyloric caeca, terminating at the tip of the papilla duodeni.

Acknowledgments

The author wishes to express her indebtedness to a number of persons for their co-operation during the course of this work. Special thanks are due to Dr. A. H. Leim, and Dr. R. H. M'Gonigle of the Atlantic Biological Station, St. Andrews, N.B., who looked entirely after the collection of materials, and to Professor A. D. Robertson of the Department of Zoology and Applied Biology, University of Western Ontario, London, Ont., who placed the requisite laboratory facilities at the writer's disposal and who very kindly undertook the photomicrography.

References

1. BALFOUR, F. M. A treatise on comparative embryology. 2nd ed. Macmillan Company, London. 1885.
2. BALLANTYNE, F. M. Trans. Roy. Soc. Edinburgh, 56(2) : 437-466. 1930.
3. CHEVEY, P. Bull. soc. zool. France, 49 : 136-145. 1924.
4. GÖPPERT, E. Morphol. Jahrb. 20 : 90-111. 1893.
5. LAGUESSE, E. J. anat. physiol. 30 : 79-116. 1894.
6. LEREBOULLET, A. Ann. sci. nat. Zool. (Sér. 4) 16 : 113-196. 1861.
7. MACALLUM, A. B. Proc. Can. Inst. (n.s.) 2(3) : 387-417. 1884.
8. MAXIA, C. Scritti Biol. 10 : 215-248. 1935.
9. POHLMANN, J. H. L. Arch. néerland. zool. 3(1) : 64-140. 1939.
10. PRICE, J. W. Ohio J. Sci. 34(6) : 399-414. 1934.
11. REIGHARD, J. Bull. Michigan Fish Comm. 1890.
12. RIGGERT, F. Beiträge zur anatomie der fischleber. Dissertation Hanover. 1922.
13. SCAMMON, R. E. Am. J. Anat. 14 : 333-390. 1913.
14. SCOTT, G. G. and KENDALL, J. I. Microscopic anatomy of vertebrates. Lea and Febiger, Philadelphia. 1935.

15. SIWE, S. A. Arch. biol. 39 : 479-509. 1929.
16. SMALLWOOD, W. M. and DERRICKSON, M. B. J. Morphol. 55(1) : 15-28. 1933.
17. STÖHR, P. A. Anat. Anz. 8 : 205-208. 1893.
18. STRICKER, F. Intern. Monatsschr. Anat. Physiol. 16 : 1-26. 1899.
19. VOGT, C. Embryologie des salmones. Histoire naturelle des poissons d'eau douce de l'Europe centrale, éd. par L. Agassiz. Imprimerie d'O. Petitpierre, Neuchatel. 1842.
20. WALLICH, C. Rept. U.S. Comm. Fisheries, 26 : 185-194. 1900 (1901).
21. WILSON, H. V. Bull. U.S. Fish, Comm. 9 : 209-277. 1889 (1891).
22. ZIEGLER, E. Die embryonale entwickelung von *Salmo salar*. Inaugural dissertation Universitäts—Buchdruckerei von Chr. Lehmann, Freiburg, I.B. 1882.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. MAY, 1942 NUMBER 5

PHYSICAL CHANGES IN THE CONSTITUENT PARTS OF DEVELOPING SALMON EGGS[1]

BY F. R. HAYES[2] AND F. H. ARMSTRONG[3]

Abstract

Wet weight and dry weight determinations were made on salmon eggs, egg capsules, detached embryos, and yolk sacs from fertilization to the end of yolk absorption. One capsule weighs 5.0 mg. wet and 2.3 mg. dry. No significant change could be found during development. Water hardening of the eggs, which occurs on transfer from coelomic fluid to water, is independent of fertilization. Increased tension of the capsule (resistance to breakage) becomes noticeable two hours after transfer to water and proceeds at a maximum rate for 30 hr. By contrast the uptake of water begins immediately on transfer from coelomic fluid and is virtually completed within an hour. It is concluded that the stoppage of water uptake is not due to the hardening of the capsule. The theory is presented that (*a*) all the water taken up becomes perivitelline fluid, (*b*) there is no loss of salt or gain of water by the yolk, (*c*) the transfer of water through the capsule is not due to osmosis but to imbibition by protein liberated into the perivitelline space by the yolk. The mortality rate for eggs reaches a maximum 14 days after fertilization (i.e., 36 days before hatching). The water content of the yolk decreases steadily during development, from 63.5 to 55.5%. Embryos from small eggs tend to compensate by growing more rapidly than those from large eggs, through the mechanism of extra water uptake. As to efficiency, which proved independent of egg size, the embryo wastes 30% of the yolk in turning the other 70% into living tissue.

This paper records weight changes and related phenomena of the constituent parts of developing salmon eggs and larvae up to the time the yolk sac is absorbed. The data are available for the interpretation of chemical analyses which have been carried out in this laboratory (10, 11) and also provide additional information regarding the problems of water uptake at the time the eggs are laid, the growth rate of the embryo, the effect of egg size on growth, and the water relations of the different parts of the system.

Methods

Fertilized and unfertilized eggs of the Atlantic salmon, *Salmo salar* L., were obtained from the Government Fish Hatchery at Bedford, N.S., at the time of spawning in November, and were maintained in a small hatchery in the university. The temperature of the tap water during November was some-

[1] *Manuscript received December 29, 1941.*

Contribution from the Zoological Laboratory, Dalhousie University, Halifax, N.S. With financial assistance from the National Committee on Fish Culture.

[2] *Associate Professor of Zoology.*

[3] *Student Assistant in Zoology.*

times as high as 10° C. or even more. From December on, the variation was only between 5.0° and 6.5° C. Owing to differences in temperature from season to season the time from fertilization to hatching and from hatching to the absorption of the yolk sac is not always the same. As a sort of norm the values presented in this paper have been corrected so as to call fertilization minus 50 days and absorption of the yolk sac about 70 days. Each batch of eggs used required a special formula for time correction, depending on the interval in the main hatchery and the temperature there, and the length of time and temperature conditions in the university. Zero time is taken as the central hatching date. Statements of time should be considered plus or minus 10%.

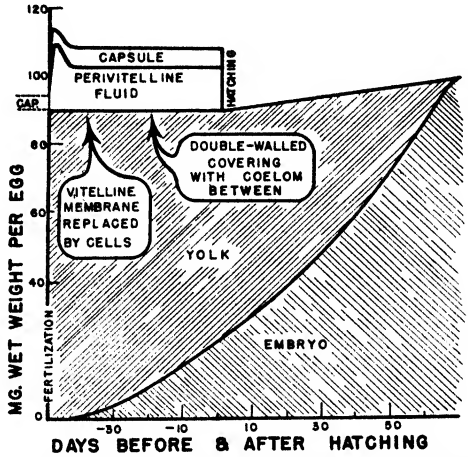


FIG. 1. *Changing relationships of the constituent parts of an egg during development.*

Fig. 1, which is based on some of the observations made, shows diagrammatically the changing relationships of the constituent parts of an egg during development. The yolk (60% water) is gradually turned into living embryonic cells (85% water). Most of the extra water comes from outside especially after hatching, so that the curve of larval weight goes up. This happens in spite of yolk used up to provide energy of maintenance, i.e., water gain is greater than yolk loss. There is no measurable exchange of fluid between the perivitelline fluid and larva, nor could there be found (except at the very beginning) any significant fluctuations in the weight or water content of the capsule. The yolk is initially covered by a fragile, non-cellular membrane, which is gradually replaced by a single layer of ectodermal cells, the process being completed by 15 days after fertilization (closure of the blastopore). This point also marks a maximum in the death rate. Some time later the coelom with its system of membranes is extended down to cover the yolk sac and by this time the eggs will stand very rough treatment without injury.

Wet weight (or fresh weight) and dry weight determinations were made throughout development on groups of 10 of each of the following: whole eggs,

capsules, larvae, yolks, and embryos. Adherent moisture was first removed with Kleenex, then if desired, the egg could be opened and the embryo lifted off the yolk with fine forceps. Drying was carried to a constant weight at 100° C. During the early part of egg development, before the coelom had covered the yolk, the living larva (yolk plus embryo) could not be removed intact from the capsule because the yolk sac ruptured. The liquid yolk made it difficult to dissect off the embryo and weigh it with any accuracy. Consequently for the early stages (up to 23 days before hatching), 5% formalin was used to harden the embryos and make handling possible. The formalin weighings were carried on to overlap the later fresh weights in order to determine the effect of fixation on weight. It was found that formalin increases the embryonic weight by some 21%, and an appropriate correction has been applied to the early weights of embryos (five sets in all). The data on the effect of formalin are in substantial agreement with those of Tunison *et al.* (23) for somewhat larger trout, of average weight 0.84 gm. These workers found that 24 hr. exposure to 10% formalin caused a weight increase of 20%, following which there was a gradual decrease until, six months later, the weight was only 11% above the original value. Tarkhan (22) tested the effect of 5 to 10% formalin on adult mammalian tissues, and was unable to find any volume change over periods of 24 to 48 hr. No yolk correction has been necessary as yolk can be measured indirectly without hardening, by the equation:

$$\text{Whole egg} - \left\{ \begin{matrix} \text{embryo} + \\ \text{perivitelline fluid} + \\ \text{capsule} \end{matrix} \right\} = \text{yolk}$$

The three items to be subtracted being small, no serious error is introduced. The errors in weighing were: earliest embryos 10 to 15%, later dropping to 2%. Perivitelline fluid could be estimated only to within 20 or 30%. Yolk sac weights show in general less than 5% error, while whole eggs or larvae could be weighed quite accurately, usually with less than 2% error.

The Egg Capsule

In the coelom of the parent fish the egg capsule was observed to be fragile, flexible, transparent, and soluble in dilute alkali as previously reported by Runnström (17) and Bogucki (2). As stated by Young and Inman (24), on transfer to water it becomes a tough, opalescent, elastic, and insoluble structure, chemically classified, in the terminology of Block, as a pseudokeratin. Its appearance is unchanged until just before hatching, at which time it ruptures due to the action of the hatching enzyme; details of this are given by Hayes (9). Weight estimations were made periodically on groups of capsules. The wet weight fluctuated between 4.8 and 5.1 mg. per capsule, but there was no evidence of consistent change during development. The dry weight remained constant. The average values per capsule are:

Wet weight = 5.0 mg.
Dry weight = 2.3 mg.
Water, % = 54

In order to see whether the weight of a capsule is related to its strength, wet and dry weights were determined for individual capsules from eggs that had been found to burst at different pressures. The greatest variation in capsular strength is found while the softening of Hein is taking place some three weeks before hatching, and it was at this time that the tests were made. (Amplification of the preceding sentence is given by Hayes (9).) No change in wet or dry weight associated with the softening of the capsule could be found.

Water Hardening

The term "water hardening" is used to describe the changes that occur in an egg when it is transferred from coelomic fluid to water. These changes, as Bogucki (2) and Manery and Irving (13) have shown, take place in the same way in fertilized and unfertilized eggs. (Bogucki states that Przylecki found in 1917 that the swelling of a frog's egg on transfer to water is also independent of fertilization.) Measurements of the two most conspicuous processes, hardening of the capsule and uptake of water have been made. The former

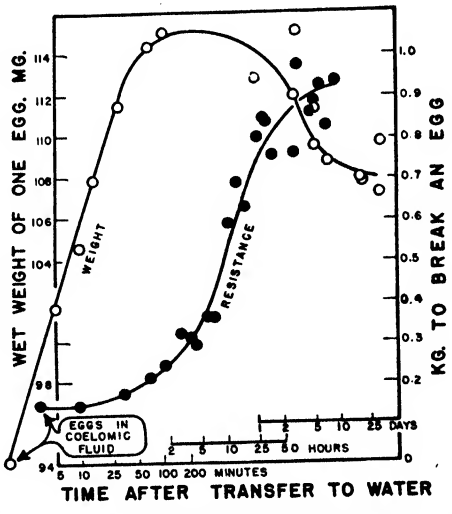
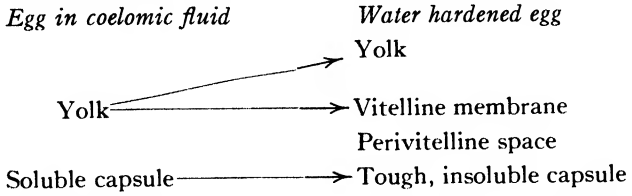


FIG. 2. *Changes in egg weight (i.e. water uptake) and in the force necessary to burst an egg during the first hours after transfer from coelomic fluid to water. Data were obtained at 10° C. The method of measuring egg resistance and the sampling errors are described by Hayes (9) where a later part of the curve is considered.*

is determined by measuring the force necessary to burst an egg, the latter by weighing live eggs at intervals. (The dry weight of an egg being found to remain constant, the weight changes are a measure of the water uptake.) The results are plotted in Fig. 2 for which semilogarithmic paper has been used in order to direct attention to the earlier values. It will be noticed that the toughening of the capsule does not become marked until some two hours after transfer to water and proceeds at a maximum rate up to about 30 hr. On

the other hand the uptake of water begins immediately on transfer and is virtually completed within one hour. Subsequently some of the added water is lost, but by the time the egg is six days old its water content is stabilized at a level that is maintained up to the time of hatching. The visible changes that take place may be summed up in the following plan.



It is here suggested that (*a*) all the water taken up by the egg becomes perivitelline fluid, (*b*) there is no loss of salt by the yolk, (*c*) therefore the transfer of water through the capsule is not due to osmosis. If the suggestion is correct the following conditions should be fulfilled.

1. The quantity of perivitelline fluid as measured directly should be the same as the increase in weight. To obtain the former an egg was first weighed, then the capsule was opened and adherent moisture was removed from capsule and larva, which were then weighed. Capsule plus larva subtracted from whole egg gives perivitelline fluid. (This test can first be done only when the eggs are half-way to hatching and the yolk has acquired a double cellular wall; in earlier attempts the yolk sac breaks.) Bogucki (2) has measured the perivitelline volume (not weight) and his results and the present ones are given in Table I. The agreement between the data of Column 2 and Column 3 is fair, and supports suggestion (*a*) of the preceding paragraph.

TABLE I

A COMPARISON OF THE INCREASE IN WEIGHT OR VOLUME OF THE WHOLE EGG, ON STABILIZATION AFTER TRANSFER TO WATER, WITH THE AMOUNT OF PERIVITELLINE FLUID AS MEASURED DIRECTLY

Material	Increase in whole egg as percentage of original value	Perivitelline fluid as percentage of original egg
Salmo fontinalis (Bogucki's results) Volume	20	23
Salmo salar Weight	20	15

2. The larval weight, when one is first able to measure it, should be the same as that of the coelomic egg less capsule. (It is assumed that the yolk used up by the embryo in the interval is too little to be measured.) The values are:

Wet weight of egg in coelom minus capsule 89.1 mg.

First measured larval weight (23 days before hatching) 90.3 mg.

The agreement, which is within the limits of variation of the material, is as it should be according to the assumption.

3. The osmotic pressure of the yolk should be unchanged by transfer to water. Svetlov (21) measured the freezing point depression of the trout yolk periodically and found it to be unchanged by water hardening, and constant up to the time of hatching.

4. The perivitelline fluid should be at all times practically equivalent in osmotic pressure to water. Svetlov (21) measured it directly and found that $\Delta = 0.01$ to 0.02.

5. The decrease in osmotic pressure of the whole mixed egg contents after transfer to water should correspond to water taken up as measured by the increase in weight. The values found for the water increase are as follows:

Water in an egg after hardening = 77.4 mg.

Water in an egg before hardening = 59.6 mg.

Difference = 17.8 mg. or 29.9% of the prehardening value. The diminution in osmotic pressure which corresponds to this added water = $\left(100 - \frac{100}{129.9}\right) = 22.9\%$ of the prehardening value. In the present work, the osmotic pressure has not been measured but the findings of others are given in Table II. It will be seen from Column 4 that while they are not inconsistent with the hypothesis, they scarcely provide conclusive proof of it. Test five is doubtfully passed.

TABLE II

THE EFFECT OF TRANSFER FROM COELOMIC FLUID TO TAP WATER ON THE OSMOTIC PRESSURE OF THE WHOLE, MIXED EGG CONTENT (THE COELOMIC FLUID IS ISOTONIC WITH THE EGGS IN IT). OSMOTIC PRESSURES ARE GIVEN AS DEPRESSION OF THE FREEZING POINT IN °C.

Material	Egg in coelomic fluid	Water hardened egg	Difference as percentage of Column 2	Observer
Salmo salvelinus	0.645°	0.599°	7.1	Runnström (17)
Salmo fario	0.497°	0.435°	12.5	Svetlov (21)
Salmo salvelinus or *S. irideus*	0.644°	0.442°	31.4	Bogucki (2)
Salvelinus fontinalis, chloride content in milliequivalents per litre	49.5	41.0	17.2	Manery and Irving (13)
Salmo salar theory requires			22.9	

It is now necessary to inquire what makes the egg take up water at the time it is laid, why there is no salt loss, and what finally stops the swelling. The most plausible explanation to date is that of Bogucki (2) whose theory is as

follows. On transfer to water the yolk liberates into the perivitelline space, colloidal substances characterized by the ability to imbibe water. The colloids cannot pass out through the capsule, but water can and does pass in. Hence imbibition is the immediate cause of the formation of the perivitelline fluid. The swelling is specifically inhibited by salt. When not so inhibited the power of imbibition is capable of surpassing the osmotic pull, even of a hypertonic solution, e.g., 0.8 *M* urea. The process comes to final equilibrium both because the power of imbibition decreases with water uptake and because the hardening of the capsule offers resistance to further swelling. Such a condition of equilibrium is illustrated in Fig. 3, in which it is assumed that the live vitelline membrane is permeable to water but not to salt. If it is impermeable to water as Gray (7) suggests, the plan becomes even simpler. If it were permeable to salt, which is unlikely, the scheme would not work. Gray (5) points out that when an egg dies, however, the covering of the yolk sac does become permeable; there is then a loss of electrolytes to the surrounding water and subsequent precipitation of the yolk globulins which are insoluble in tap water.

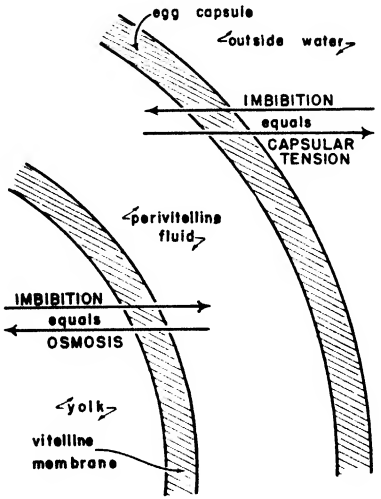


FIG. 3. *A diagram suggesting the mechanism that brings about water equilibrium a few days after the eggs are laid, i.e., transferred from coelomic fluid to water.*

In support of Bogucki's theory there are several pieces of evidence which will be briefly summarized.

1. According to Svetlov (21) and Bogucki (2) the capsule after water hardening, and presumably before, is permeable in either direction to water, to such salts as potassium, sodium, calcium, and mercuric chloride and potassium cyanide, to such simple organic molecules as urea, glucose, and lactose, to the dyes neutral red, Nile blue sulphate, crystal violet, eosine, and auramine, and to the amino acids, glycocoll, phenylalanine, and leucine. On the

other hand the capsule is impermeable, or practically so, to such colloidal solutions as starch, trypan blue, India ink, Congo red, and egg albumen. Svetlov's experiments, especially with salt solutions, have been questioned by Hayes (8) on grounds of inadequate technique. The criticisms do not, in the main, apply to Bogucki's work and the above list may now be provisionally accepted.

2. Those dyes that do go through the capsule will not go into the yolk (1).

3. The perivitelline fluid contains protein. It is slightly opalescent in appearance, and is clearly positive to Millon's reaction in later stages, less clearly at the start (21). Before the theory can be finally accepted there will have to be direct proof that enough protein exists in the fluid to imbibe the quantities of water which are found there.

4. As noted above the perivitelline fluid contains practically no salt.

5. Verification has been made of Runnström's (17) observation that the swelling of the egg is inhibited by sea water dilutions in direct proportion to their osmotic pressure until at the point of isotonicity there is no swelling. Runnström has further shown that solutions of single salts, as well as Ringer's solution, inhibit swelling. Nevertheless the swelling is not inhibited even in hypertonic solutions of glucose or urea (2). For this there might be two explanations between which no decision can be made at present. (*a*) The inhibition is a specific salt effect rather than an osmotic phenomenon (Bogucki's explanation). (*b*) Many cell membranes (that of the erythrocyte is a well known example) are found to be freely permeable to urea and glucose while quite impermeable to salts, so that the salt solutions, but not the urea solutions, exert osmotic pull. If the vitelline membrane exhibited such a property the results could be readily explained as an osmotic effect.

The Effect of Age on Mortality

It is a hatchery man's rule of thumb that eggs can be handled quite roughly for about 36 hr. after fertilization, but following that time they must be disturbed as little as possible until the "eyed stage" is reached, i.e., almost half-way to hatching. It is clear that there is a period of great susceptibility in the early part of the egg stage. By way of establishing the point of maximum delicacy, the death rate of normally developing eggs was observed from day to day. The total losses amounted to about one-quarter of the eggs, and the daily losses are plotted in Fig. 4. The data for other batches of eggs are similar. It will be seen that the maximum is very striking, and occurs just under one-third of the way to hatching, or in the present case, 14 days after fertilization. In 1906, Steuert (20) measured the resistance of trout eggs to "mechanische Insulte". He found the time of maximum susceptibility to be in the period 10 to 17 days after fertilization (the eggs took 50 days to hatch). In the following year Hein (12) published the results of similar and more extensive experiments also on trout; these showed 15 days after fertilization to be the most critical time for the eggs (54 days to hatch).

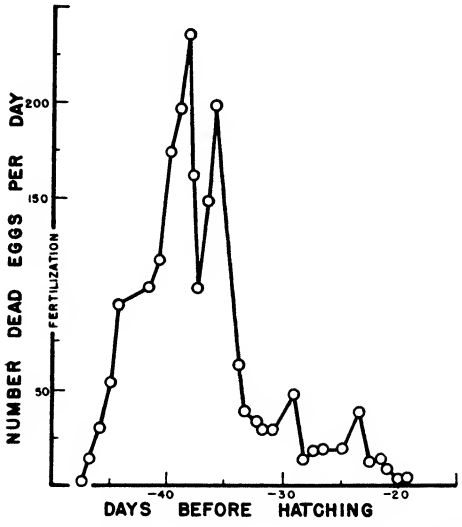


FIG. 4. *Variation in the death rate of undisturbed eggs during development.*

Thus the present results confirm those of Steuert and Hein. The period of maximum death rate corresponds to the closure of the blastopore, i.e. the vitelline membrane over the yolk is replaced by a layer of cells. Hence the immediate cause of death in early stages may be rupture of the vitelline membrane. It has often been noticed that in dead eggs the membranes seem to have slipped up and formed a crumpled mass at the top. It is natural to think that the closure of the blastopore would make this less likely to happen. Still later in egg development the yolk sac becomes very heavily protected by a composite layer of ectoderm, somatic mesoderm, coelom, splanchnic mesoderm, and endoderm. By this time the eggs will stand very rough handling.

In the cod egg it appears that the period of maximum susceptibility is rather earlier and has already passed by the time the blastopore has closed (16, 3).

In the early mortality period just described the sign of death is a general whitening of the egg. Another period of major loss occurs shortly before hatching, and here it is the embryo and not the yolk which first becomes opaque (details in 8, pp. 723–4 to which may be added the following new observations). First, the death rate is greatly accelerated by increased temperature, second, it can be abolished by premature hatching of the eggs. Actually eggs at 13° to 14° C. which were at the point of death were brought to recovery by artificial hatching. Third, eggs carried through at 5° to 6° C. from the beginning cannot stand transfer to higher temperatures shortly before hatching, although it is well known that salmon eggs can be reared at 10° C. and even more. (Do the latter eggs give rise to smaller embryos?)

The explanation previously suggested was that the embryo was adversely affected by the hatching enzyme or some product of it. It is now known (9)

that although the capsule begins to soften some weeks before hatching, the hatching enzyme itself appears less than a day before the egg bursts. The adverse effects on the embryo (white spots, etc.) may appear a fortnight or more before hatching, and it is therefore unlikely that they are associated with the hatching enzyme, although they may still be related in some way to the softening of the capsule. There is also a possibility that death at higher temperatures may be due to an insufficient diffusion of oxygen through the capsule to meet the needs of the already well grown embryo. The rate of oxygen diffusion is practically unaffected by temperature while the embryonic consumption is increased three- or fourfold by a rise of 10° C. If the embryo were using all the oxygen it could get at lower temperatures, there would not be enough to keep it alive at higher temperatures. The idea of oxygen lack as a cause of death is made plausible by some recent work of Moore (14), who states "Those species of frogs [e.g. *Rana sylvatica*] breeding early in the spring when the water is cold have a submerged compact jelly mass. The closely crowded eggs, however, die of asphyxiation at temperatures such as those existing in the environment in which [the summer breeding forms] *Rana clamitans* and *Rana catesbeiana* breed. The latter two species deposit their eggs in a surface film that insures a better supply of oxygen." It is of interest that the death of the eggs of *R. sylvatica* at 25° C. can be prevented by substituting oxygen for air.

The Larva

A larva is an embryo plus its attached yolk sac. It is in this form that hatching takes place. In Fig. 5 which shows the fluctuation in dry weight and water content, two features are very clear, the increase in water and the decrease in dry weight that occur after hatching. The former is a measure of the environmental water taken up by the embryo. There is no clear indication of water uptake before hatching, which suggests that the salmon egg is a self-

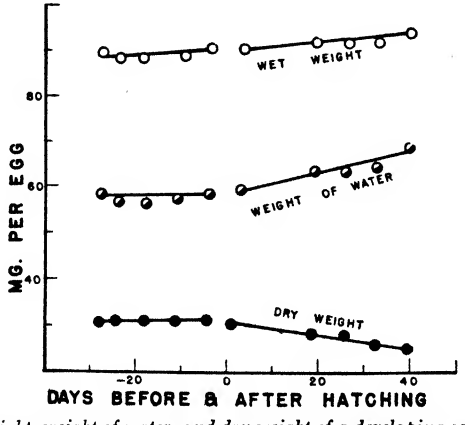


FIG. 5. *Total weight, weight of water, and dry weight of a developing salmon larva (embryo plus yolk). For observations previous to hatching the egg capsules were torn open and the larvae removed.*

contained system. However, some evidence to be presented below makes it probable that when eggs are smaller than is characteristic for the species, the embryos (which are also too small at the start) may compensate by taking in water from the environment earlier than usual, i.e. before hatching. The lower curve of Fig. 5, which begins to drop immediately after hatching, is a measure of the amount of material used up by the developing embryo. Combustion of yolk provides energy for wriggling and swimming, for the basal metabolism of the living material already there, and for new construction. All these demands increase rapidly after hatching. No drop in the dry weight of the larva can be shown in the interval before hatching. This does not mean, of course, that the embryo is developing with 100% efficiency, but merely that the loss that does occur is too small to be measured with a balance. The loss can readily be demonstrated by respiratory measurements.

The Water Content of the Yolk Sac

The percentage of water in the yolk decreases steadily during development. Of about 25 estimates made, a few will serve to illustrate the trend.

Percentage of water (calculated):

At the start	63.5
Half-way toward hatching	62
At hatching time	59
Yolk sac half absorbed	55.5

The conclusions are that the embryo is taking relatively more water than dry material from the yolk, and that this process is unchanged by hatching. Scheminzky (18) reports a rise in the water content of the yolk sac after hatching, which the writers have been unable to confirm. With regard to the first value "at the start", from evidence already presented it is believed that it would be the same before and after water hardening.

The Embryo

It is proposed to present in this paper only a description of the effect of egg size on embryo size and growth.

For the purpose two sets of eggs from two females were reared under identical conditions. There were eggs of adult salmon, averaging in early stages 108.5 mg. each, and eggs of grilse (salmon spawning for the first time) which weighed 79.0 mg. each. Fig. 6 shows the wet weights of grilse and salmon embryos (removed from yolk) plotted against time. It is evident that the grilse embryos, though smaller to start with, are tending to approach those of the salmon in wet weight. When the logarithms of the wet weights are plotted against time, as in Fig. 7, it becomes clear that the growth rate of the grilse embryo exceeds that of the salmon in the period under consideration. The lines drawn have not been placed by eye, but by calculation from all the points, and it can be shown that the slopes differ significantly.

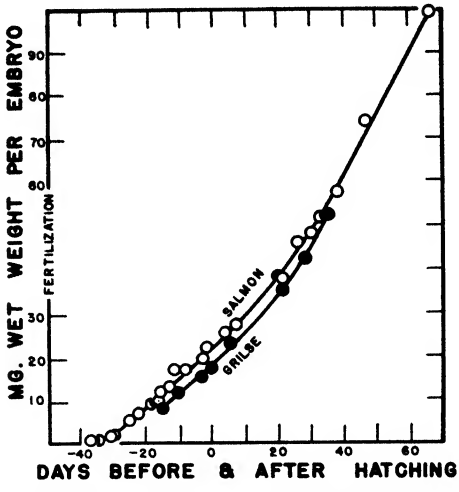


FIG. 6. *Effect of egg size on the growth of the embryo only (not larva). Upper curve eggs from adult salmon, lower curve smaller eggs of grilse (i.e., salmon spawning for the first time).*

These results show that although the grilse embryo is smaller than the salmon embryo at the beginning of development, by 35 days after hatching it is approximately the same size as the salmon. It is reasonable to assume that the initial smallness of the grilse embryo is related to egg size. A possible explanation is that there is a smaller amount of protoplasm at the beginning of development and that, therefore, the embryo formed is smaller. Little is known about the method of absorption of materials from the yolk sac but it may be that in the early stages it bears some relation to the surface of the embryo on the yolk sac. Therefore, one might say that it is not until the development of the vitelline circulation that the grilse embryo is in a position

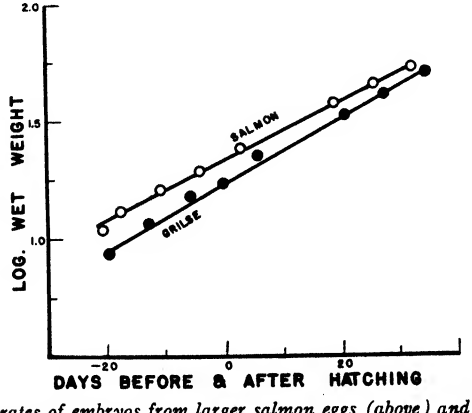


FIG. 7. *Growth rates of embryos from larger salmon eggs (above) and smaller grilse eggs. The slopes of the lines can be shown to differ significantly.*

to withdraw the maximum amount of yolk sac material. Then although there is much more yolk in the yolk sac of the salmon, the grilse embryo begins to catch up to the salmon.

A general phenomenon observable in all forms, is the characteristic species size. Marked differences in the young do not prevent them from approaching the adult size of their species. No one would suppose that a grilse embryo would in five years be very different in size from any other five year salmon. The interesting thing about this investigation is that this tendency shows itself in the salmon at such an early age. It is undoubtedly true that a grilse embryo will have used up its supply of yolk before the salmon embryo, but it is then quite capable of utilizing external food. The salmon embryo will, however, be larger before it has to search for external food and will therefore have a greater chance of surviving.

Byerly (4) has found that in the chick there is no demonstrable difference in growth rates of embryos from eggs of different sizes between the 3rd and 18th days, i.e., when his results are plotted in the manner of Fig. 7 the straight lines are parallel. After the 18th day the rates diverge owing to lack of food in the small eggs. There are some obvious differences in the development of the salmon and chick. The former hatches while still in the yolk sac stage and is able to take up water from its environment, but the chick must be supplied with sufficient water to last until hatching, which takes place at a much later morphological stage. Moreover a salmon, although provided with relatively little yolk, is a free-swimming form able to seek external food long before the yolk sac has disappeared. In the chick on the other hand the smallness of the yolk will be felt in the days before hatching.

Dry weights of salmon and grilse embryos were also observed and it was found that the rate of increase of dry material showed no significant difference in the two cases. Thus, wet growth of grilse is greater than that of salmon; dry growth rates are not significantly different; therefore the extra growth rate of the grilse is due to water uptake.

Mention may here be made of the fluctuations in the water content of the embryo. In the earliest observations the water was just under 85% and it remained there until about half-way to hatching, when it rose rather suddenly to a maximum of 91%. The sudden rise (which is paralleled in the corresponding stage of the chick, according to Schmalhausen (19)) may be due to the appearance of lymph-filled cavities such as the optic cup, brain ventricles, coelom, and auditory vesicle, as well as to the development of the circulatory system. From −26 days on through hatching to the end of observations the water content decreased steadily. By the last observation at 47 days (the yolk sac had nearly disappeared) the percentage of embryonic water was 82.7. As the preceding paragraph implies, the percentage of water does not drop as rapidly in the grilse as in the salmon, i.e., the grilse embryo, having less dry yolk to draw on, tends to bring its weight up by water.

The water content of developing trout embryos has been reported as constant by Gray (6). According to Scheminzky (18) the percentage of water in the

trout falls before hatching and then rises to a constant level after hatching. The present results differ from both these reports.

It is of interest to determine the source from which the embryo obtains the large quantity of water needed for its development. Some of it, as is already known, comes from the yolk, which becomes steadily more dry as development goes on. But when does outside water begin to supplement this diminishing supply? The answer is given in Fig. 8 which shows that the salmon embryo

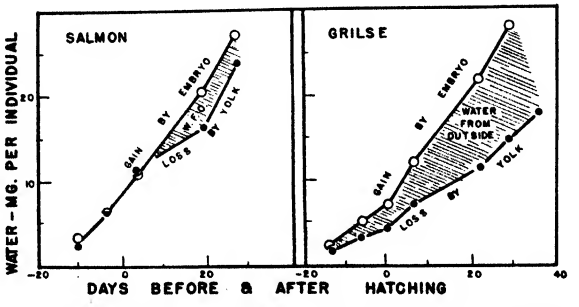


FIG. 8. *A comparison of the water taken from outside the yolk sac by the embryos of salmon and grilse.*

does not begin to take up water until after hatching. The grilse embryo, on the contrary, compensates for its small egg stores by taking up, after hatching, a good deal more water than the salmon embryo, and also, apparently, by beginning to supplement the yolk water even before hatching. Unfortunately the observations on prehatching grilse are too few in number to stand up to a statistical examination, so that an uptake of water before hatching has not been proved. There is, however, other evidence for water uptake before hatching in fish eggs and other eggs, which is reviewed by Needham (15, pp. 906–911).

The dry weight consumed by the embryo, which is shown by the drop in the bottom line of Fig. 5, may also be calculated as the difference between the loss in dry yolk and the gain in dry embryonic tissue. This is plotted for the salmon and grilse in Fig. 9. As might be expected the salmon embryo, being larger, uses up somewhat more material than the grilse embryo. A more instructive comparison is obtained if the efficiency of the process is calculated by the equation:

$$\frac{\text{Dry weight gained by embryo} \times 100}{\text{Dry weight lost by yolk}}$$

It is not possible to give any useful values for the interval before hatching, because the changes in weight are so small in comparison with the total weight that the errors become very large. The calculations, therefore, have been restricted to the last five sets of points (to the right) on each curve. For the four intervals between them the embryo gain and yolk loss is obtained by subtraction, and the efficiency calculated. Two facts emerge. First, there

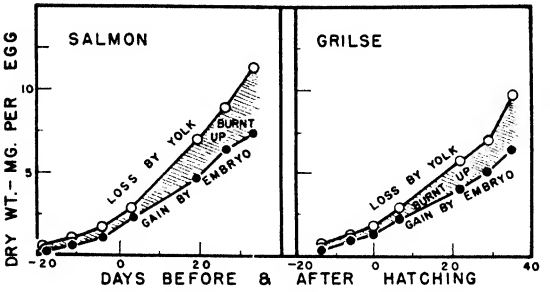


FIG. 9. *A comparison of the growth in dry weight of the embryo with the loss in dry weight of the yolk, in salmon and grilse.*

was no indication in either case of a trend towards increased or decreased efficiency with time. Second, there was no measurable difference in efficiency between the salmon and grilse embryos. The probable errors were about $\pm 8\%$ of the values given; the values were on the average:

Efficiency of the salmon embryo	68%
Efficiency of grilse embryo	70%

Stated otherwise these figures mean that in the interval under consideration some 30% of the yolk is wasted in the process of turning the other 70% into living embryo. Previous studies of embryonic efficiency in salmon and trout have led to conflicting results with values ranging from 43 to 63% in the stages after hatching. The subject is reviewed by Needham (15, pp. 934–939).

Acknowledgments

It is a pleasure to make acknowledgment to Dr. D. Pelluet for the use of unpublished data on salmon embryology, and also to Dr. D. Mainland for advice on statistical tests.

References

1. ADLER, P. Protoplasma, 15 : 15-23. 1932.
2. BOGUCKI, M. Protoplasma, 9 : 345-369. 1930.
3. BONNET, D. D. Biol. Bull. 76(3) : 428-441. 1939.
4. BYERLY, T. C. J. Exptl. Biol. 9(1) : 15-44. 1932.
5. GRAY, J. J. Physiol. 53 (5) : 308-319. 1920.
6. GRAY, J. Brit. J. Exptl. Biol. 4(2) : 215-225. 1926.
7. GRAY, J. J. Exptl. Biol. 9(3) : 277-299. 1932.
8. HAYES, F. R. Biochem. J. 24(3) : 723-734. 1930.
9. HAYES, F. R. J. Exptl. Zool. In press. 1942.
10. HAYES, F. R. and HOLLETT, A. Can. J. Research, D, 18(2) : 53-65. 1940.
11. HAYES, F. R. and ROSS, D. M. Proc. Roy. Soc. (London) B, 121 : 358-375. 1936.
12. HEIN, W. Allgem. Fischerei-Ztg. 32 : 383-387, 398-402. 1907.
13. MANERY, J. F. and IRVING, L. J. Cellular Comp. Physiol. 5(4) : 457-464. 1935.
14. MOORE, J. A. Am. Naturalist, 74 (750) : 89-93. 1940.
15. NEEDHAM, J. Chemical embryology. Cambridge University Press. 1931.
16. ROLLEFSEN, G. J. conseil intern. exploration mer, 7(3) : 367-373. 1932.
17. RUNNSTRÖM, J. Acta Zool. Stockholm, 1 : 321-336. 1920.

18. SCHEMINZKY, F. Arch. ges. Physiol. (Pflüger), 223(4 and 5) : 645-656. 1929.
19. SCHMALHAUSEN, I. Arch. Entwicklungsmech. Organ. 108(2) : 322-387. 1926.
20. STEUERT, L. Naturw. Z. Forst.-u. Landw. 4 : 92-96. 1906.
21. SVETLOV, P. Arch. Entwicklungsmech. Organ. 114(4 and 5) : 771-785. 1929.
22. TARKHAN, A. A. J. Roy. Microscop. Soc. 51(4) : 387-400. 1931.
23. TUNISON, A. V., PHILLIPS, A. M., MCCAY, C. M., MITCHELL, C. R., and RODGERS, E. O. Cortland Hatchery Rept., New York State Conservation Dept. 8. 1939.
24. YOUNG, E. G. and INMAN, W. R. J. Biol. Chem. 124(1) : 189-193. 1938.

FIELD TRIALS OF CONTROL MEASURES FOR PARASITIC DISEASES OF SHEEP[1]

By W. E. Swales[2]

In collaboration with

P. E. Sylvestre[3] and S. B. Williams[4]

Abstract

Field tests of methods of helminth parasite control through anthelmintic medication of sheep flocks on the ranges of the Central Experimental Farm, Dominion Department of Agriculture, Ottawa, during 1937, 1938, 1940, and 1941 are described briefly.

Some observations on the effects of parasite burdens have been made.

Following the development of phenothiazine in 1939 as a practical and highly effective anthelmintic for sheep, medication of adult sheep before the pasturing season was very effective in preventing nodular worm infections in lambs. One early spring treatment in 1940 and a similar one in 1941 reduced the incidence of nodular disease lesions to 0.65 per lamb, a reduction from the figures of 1938 of 99.1% in the lambs slaughtered in the autumn.

In spite of no treatment of the lambs during 1940 and 1941, *Haemonchus*, *Monodontus*, and *Cooperia* were reduced to very low numbers and no significant increase in other genera occurred.

It is suggested that *Oesophagostomum* has been reduced to a level too low for subsequent recovery in these flocks unless it is reintroduced in new stock; thus this parasite can be considered as probably eradicated.

In sheep flocks used for experimental work, particularly in tests to determine the nutritive value of pastures, disease due to worm parasites is a serious factor which cannot easily be measured. For this reason, a co-operative project between the Institute of Parasitology and the Division of Animal Husbandry, Central Experimental Farm, Ottawa, was arranged in 1937. The objects of this project were to test the efficacy of various routine treatments used to control infections with various worm parasites and at the same time to determine the relative importance of certain species. As the work progressed, independent researches on the bionomics of certain species and critical tests of newer anthelmintics were conducted at the Institute of Parasitology and a system of prevention of parasitic disease was evolved for the conditions encountered in Eastern Canada. This system has now been extended to many sheep raising regions.

The extent of the work makes it necessary to present this paper in a very much condensed form, particularly in view of the need for economy. However,

[1] *Manuscript received April 4, 1942.*

Contribution from the Division of Animal Pathology, Science Service, the Division of Animal Husbandry, Experimental Farms Service, Dominion Department of Agriculture, and the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Associate Animal Pathologist, Science Service, Dominion Department of Agriculture.*

[3] *Agricultural Scientist (Beef cattle and sheep investigations), Division of Animal Husbandry, Experimental Farms Service, Ottawa.*

[4] *Graduate Assistant, Division of Animal Husbandry, Experimental Farms Service, Ottawa. (Now on active service overseas.)*

further details are available at the Institute of Parasitology for workers who might require them at any time.

The work described here was divided into various phases as data were collected. In 1937, tests of five treatments were made; three were well known anthelmintic treatments (as originally developed by Curtice (1), Hall and Foster (4), and Hall and Shillinger (5)), one was a modification of a new treatment for nodular worm infection devised in South Africa by Mönnig (7), and the fifth was a new method of administration of capsules of tetrachlorethylene. A flock consisting of approximately 300 ewes and young lambs was used; some of the ewes were from western range flocks of Rambouillets and Corriedales, and others were grade Shropshires. The lambs were their offspring, sired by Leicester or Shropshire rams. The flock was divided into five groups at random, but they remained intermingled on pasture. At the initiation of the test on June 23rd, 1936, the lambs had received no previous treatment, but the ewes had received a drench of 3 oz. of a 1% solution of copper sulphate containing 0.6% nicotine sulphate on May 12th at the time they were turned to pasture. Treatments were administered four times during the season; on June 23rd, when the lambs were about eight weeks old, on August 5th, on September 10th, and on October 21st. Lambs that were ready for market at the time of treatment were slaughtered four or five days afterwards so that only the parasites that survived the medication would be detected at autopsy. No further treatments were administered after the end of the pasturing season, but the small numbers of survivors were slaughtered in four lots on December 21st, January 14th, February 16th, and March 16th. It was thought that any clinical parasitic disease would be detected in these animals which matured slowly.

The animals were dosed, without prior fasting, and they were returned to pasture immediately. The treatments were as follows:—

Date	Animals	Treatment
Group *A*—copper sulphate solution		
23/6/37	Ewes	100 cc. 1% solution
	Lambs	40 to 50 cc. 1% solution
5/8/37	Ewes	50 cc. 2% solution
	Lambs	25 cc. 2% solution
10/9/36 and 21/10/37	Ewes	50 cc. 2% solution
	Lambs	34 to 50 cc. 2% solution
Group *B*—Cunic, a mixture of copper sulphate solution and 40% nicotine sulphate		
23/6/37	Ewes	100 cc. 1% $CuSO_4$ plus 0.65% nicotine sulphate
	Lambs	30 cc. 1% $CuSO_4$ plus 0.65% nicotine sulphate
5/8/37	Ewes	100 cc. 1% $CuSO_4$ plus 0.65% nicotine sulphate
	Lambs	50 cc. 1% $CuSO_4$ plus 0.65% nicotine sulphate
10/9/36 and 21/10/37	Ewes	50 cc. 2% $CuSO_4$ plus 1.3% nicotine sulphate
	Lambs	35 to 40 cc. 2% $CuSO_4$ plus 1.3% nicotine sulphate

Date	Animals	Treatment
Group *C*—soft gelatine capsules of tetrachlorethylene, administered by means of a modified "balling gun" syringe, which simultaneously sprayed the pharynx with 5 cc. of a 5% solution of $CuSO_4$		
23/6/37	Ewes Lambs	5 cc. C_2Cl_4 2.5 cc. C_2Cl_4
5/8, 10/9, and 21/10/37	Ewes Lambs	5 cc. C_2Cl_4 5 cc. C_2Cl_4
Group *D*—soft gelatine capsules of tetrachlorethylene, given by hand without stimulation of oesophageal groove		
As in Group *C*	As in Group *C*	As in Group *C*
Group *E*—a powder mixture of copper arsenate, 2 parts, calcium hydroxide. 3 parts, and copper tartrate, 5 parts, kept suspended by agitation in 1% $CuSO_4$ solution and administered as a drench		
24/6/37	Ewes Lambs	2.5 gm. powder in 50 cc. 1% $CuSO_4$ 50 cc. 1% solution $CuSO_4$
5/8/37 and 10/9/37	Ewes Lambs	As above 1 gm. powder in 25 cc. 1% $CuSO_4$
21/10/37	Ewes Lambs	As above 1.2 gm. powder in 25 cc. 1% $CuSO_4$

No toxic effects were noted during the course of the tests and no deaths occurred in the flock. As the speed of development was the criterion of thriftiness in the lambs, the distribution of groups in the lots marketed for slaughter as fat lambs is of interest; this distribution is shown in Table I. As the animals were chosen for market when they reached approximately 80 lb. in weight, and without regard to the group to which they belonged, the evenness of distribution is remarkable, and shows no superiority of any one of the treatments in aiding lambs to reach market grades.

TABLE I

NUMBER OF LAMBS FROM EACH GROUP (1937) MARKETED FOR SLAUGHTER

Date	Group *A*	Group *B*	Group *C*	Group *D*	Group *E*
10/8/37	2	2	2	1	2
14/9/37	11	5	7	10	5
26/10/37	10	9	12	10	13
21/12/37	2	2	0	3	1
14/1/38	1	1	1	1	1
16/2/38	3	4	2	3	3
16/3/38	1	1	2	1	2
Totals	30	24	26	29	27

Immediately after slaughter the abomasa and intestines were cooled and later shipped to the laboratory for detailed examination. The parasites were counted by methods previously described (12) and the viscera were examined for abnormalities. No heavy or harmful infections were found in the lambs, probably due, in part at least, to an abundance of pasture and the fact that the ewes recently came from western areas noted for freedom from the worms that are common in eastern Canada. The results, together with those obtained in 1938, are presented in a brief form in Tables III to XI. They are in the form of comparisons of numbers of surviving parasites and do not infer absence or presence of clinical parasitic disease.

In the cases of *Moniezia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Strongyloides*, *Chabertia*, *Oesophagostomum*, and *Trichuris* there was no significant difference between any two groups.

Haemonchus, judging by the small infections present in all the animals, was apparently removed fairly effectively by all treatments. However, "Cunic" and tetrachlorethylene were significantly more effective than the arsenate and copper preparation.

In the case of *Ostertagia*, tetrachlorethylene in capsules by simple administration was significantly more effective than each of the three drenches.

Monodontus was more effectively removed by Cunic and by tetrachlorethylene than by copper sulphate. However, the other two treatments did not show any superiority.

Procedure in 1938

During this year the same flock of ewes and their 1938 offspring were used for continuation of the tests and for continued observations on seasonal fluctuations of the worm species on the same pasture land. The ewes were treated in April with the 2% Cunic solution and went to pasture with their lambs shortly afterwards. On June 3rd, the flock was divided at random into four groups, one to remain as a control with no further medication. As in 1937 the groups were admixed on the same permanent pasture. The treatments used were a 5% Cunic drench, tetrachlorethylene in capsules, and a tetrachlorethylene emulsion drench which was previously devised and tested on a small scale at the Institute of Parasitology, and later tested by Gordon and Whitten (3). The emulsion was made by making a solution of 10 cc. triethanolamine in 250 cc. of water and slowly adding and emulsifying a mixture of 100 cc. of tetrachlorethylene, 150 cc. light liquid petrolatum, and 37.5 cc. oleic acid; thus in 53.75 cc. of the emulsion there was 10 cc. of tetrachlorethylene.

The ewes did not receive the experimental anthelmintics, but were dosed on June 3, and September 15 with 2% Cunic solution in order to avoid haemonchosis. The lambs were treated as follows:—

Date	Treatment
Group *F*—5% Cunic solution (5% $CuSO_4$ and 5% nicotine sulphate)	
3/6/38	5 cc. of 5% Cunic drench
21/7/38, 16/8/38*, 15/9/38, and 2/11/38	10 cc. of 5% Cunic drench
Group *G*—tetrachlorethylene (in capsules)	
3/6/38	2.5 cc. C_2Cl_4
21/7/38, 16/8/38*, 15/9/38, and 2/11/38	5 cc. C_2Cl_4
Group *H*—tetrachlorethylene emulsion	
3/6/38	20 cc. of 2% Cunic (considered too young for new treatment)
21/7/38	15 cc. to 25 cc. C_2Cl_4 emulsion (2.8 to 4.7 cc. C_2Cl_4)
16/8/38*, 15/9/38, and 2/11/38	25 cc. C_2Cl_4 emulsion (4.7 cc. C_2Cl_4)
Group *I*—lambs untreated	

* *On this date only the few lambs ready for market were treated.*

No ill effects were noted during the season and the flocks remained in good health. The lambs that had reached a weight of 80 lb. at the time of each treatment were marketed for slaughter from three to seven days afterwards. The distribution of groups in the market lots is shown in Table II. Group *H* (tetrachlorethylene emulsion) appeared to be slightly retarded in development, as 13 lambs remained on November 2nd as compared with 7, 6, and 6, in the other groups.

TABLE II

NUMBER OF LAMBS FROM EACH GROUP (1938) MARKETED FOR SLAUGHTER

Date	Group *F*	Group *G*	Group *H*	Group *I*
27/7/38	5	6	10	6
19/8/38	6	7	4	7
22/9/38	16	13	9	17
7/11/38	5	5	10	4
10/1/39	2	1	3	2
Totals	34	32	36	36

The viscera were studied in detail, and the results showing the number of parasites that survived the treatments are presented in Tables III to XI.

TABLE III

STOMACH WORM (*Haemonchus contortus*)

Group	Year	No. lambs	Total worms	Mean No. worms	S.D.	Test of significance			
						Groups compared	Diff. means	S.E. diff. means	Interpretation
A	1937	30	87	2.9	7.8	*A* vs. *E*	12.3	6.5	N.S.
B	1937	24	7	0.3	0.7	*B* vs. *A*	2.6	1.4	N.S.
						B vs. *C*	1.1	0.6	N.S.
						B vs. *E*	14.9	6.3	S.
C	1937	27	38	1.4	2.8	*C* vs. *A*	1.5	1.5	N.S.
						C vs. *E*	13.8	6.3	S.
D	1937	29	55	1.9	5.5	*D* vs. *A*	1.0	1.7	N.S.
						D vs. *E*	13.3	6.4	S.
E	1937	27	401	15.2	32.8				
F	1938	33	507	15.4	37.9	*F* vs. *I*	38.9	13.3	S.
G	1938	32	241	7.5	14.0	*G* vs. *F*	7.9	7.0	N.S.
						G vs. *H*	3.4	3.5	N.S.
						G vs. *I*	46.8	11.8	S.
H	1938	36	391	10.9	15.0	*H* vs. *F*	4.5	7.0	N.S.
						H vs. *I*	43.4	11.8	S.
I (Control)	1938	36	1956	54.3	69.3				

NOTE: *Anthelmintics used were as follows (Tables III to XI):—*

Group *A* — *$CuSO_4$, 2%.*
B — *Cunic, 2%.*
C — *C_2Cl_4 capsules with $CuSO_4$ spray.*
D — *C_2Cl_4 capsules alone.*
E — *Mönnig's arsenic and copper mixture suspended in $CuSO_4$.*
F — *Cunic 5%.*
G — *C_2Cl_4 capsules alone.*
H — *C_2Cl_4 emulsion.*
I — *Untreated.*
S. — *Significant (P = .05, or odds at least 19 to 1).*
N.S. — *Not significant.*

There was no significant difference in any group in the numbers of members of the following genera, *Moniezia*, *Strongyloides*, *Chabertia*, *Oesophagostomum*, and *Trichuris*.

Haemonchus was significantly higher in number in the control group than in each of the other groups, but no superiority of one treatment over another was shown.

Ostertagia was removed to a significant degree by tetrachlorethylene in capsules and in emulsion. Cunic had no significant effect on this parasite. This finding agreed with the results of 1937.

Monodontus was affected by all three treatments, but there was an apparent superiority of tetrachlorethylene in capsules over the emulsion. In calculating

TABLE IV

BROWN STOMACH WORM (*Ostertagia circumcincta*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	38,100	1270	1401				
B	1937	24	16,980	707	604	*B* vs. *A*	563	284	N.S.
C	1937	27	15,690	581	1405	*C* vs. *A*	689	372	N.S.
D	1937	29	10,510	362	600	*D* vs. *A* *D* vs. *B* *D* vs. *C* *D* vs. *E*	908 345 219 709	279 166 292 311	S. S. N.S. S.
E	1937	27	28,930	1071	1512				
F	1938	34	32,090	944	1203	*F* vs. *I*	1035	574	N.S.
G	1938	32	16,450	514	734	*G* vs. *F* *G* vs. *I*	430 1465	240 551	N.S. S.
H	1938	36	28,950	804	884	*H* vs. *I*	1175	556	S.
I	1938	36	71,240	1979	3216				

TABLE V

SMALL STOMACH WORM (*Trichostrongylus axei*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	5170	172	220				
B	1937	24	2120	88	118	*B* vs. *A* *B* vs. *C*	84 104	47 64	N.S. N.S.
C	1937	27	5180	192	307				
D	1937	29	4150	143	205				
E	1937	27	4220	156	191				
F	1938	34	5460	161	190	*F* vs. *I*	73	48	N.S.
G	1938	32	3860	121	132	*G* vs. *H* *G* vs. *I*	79 113	41 42	N.S. S.
H	1938	36	7220	200	199	*H* vs. *I*	34	36	N.S.
I	1938	36	8440	234	207				

TABLE VI

TAPEWORM (*Moniezia expansa*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1397	30	461	15.7	24.2				
B	1937	24	198	8.2	12.3	*B* vs. *A*	7.5	5.1	N.S.
C	1937	27	289	10.7	13.0				
D	1937	29	246	8.5	11.7				
E	1937	27	256	9.4	12.2				
F	1938	34	274	8.1	14.1				
G	1938	32	678	21.2	42.5				
H	1938	36	279	7.7	12.1	*H* vs. *G* *H* vs. *I*	13.5 3.6	7.8 3.2	N.S. N.S.
I (Control)	1938	36	407	11.3	15.1				

TABLE VII

HOOKWORM (*Monodontus trigonocephalus*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	742	24.7	18.1				
B	1937	24	330	13.7	12.1	*B* vs. *A* *B* vs. *E*	11.0 5.1	4.1 3.7	S. N.S.
C	1937	27	430	15.9	17.5	*C* vs. *A*	8.8	4.7	N.S.
D	1937	29	438	15.1	15.3	*D* vs. *A*	9.6	4.4	S.
E	1937	27	508	18.8	14.1	*E* vs. *A*	5.9	4.3	N.S.
F	1938	34	126	3.7	3.8	*F* vs. I_2	2.4	1.2	S.
G	1938	32	71	2.2	2.5	*G* vs. *F* *G* vs. *H* *G* vs. *I* *G* vs. I_2	1.5 3.2 7.0 3.9	0.8 0.8 19.6 1.1	N.S. S. N.S. S.
H	1938	36	193	5.4	4.3				
I (Control)	1938	36	333	9.2	117.6				
I_2 (Control)	1938	35	213	6.1	6.1				

TABLE VIII

THIN-NECKED THREADWORM (*Nematodirus* spp.)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	28,380	946	1385				
B	1937	24	19,690	820	962				
C	1937	27	13,570	503	602				
D	1937	29	11,840	408	539	*D* vs. *A* *D* vs. *B*	538 412	272 220	N.S. N.S.
E	1937	27	22,620	838	1077				
F	1938	34	46,720	1374	2419	*F* vs. *I*	1083	828	
G	1938	32	31,030	970	1363	*G* vs. *I*	1487	756	
H	1938	36	22,940	637	923	*H* vs. *F* *H* vs. *I*	737 1820	442 733	N.S. S.
I (Control)	1938	36	88,470	2457	4302				

TABLE IX

Cooperia spp.

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	26,590	886	1071				
B	1937	24	27,010	1125	1443				
C	1937	27	30,300	1122	1817				
D	1937	29	24,820	856	1248	*D* vs. *B*	270	375	N.S.
E	1937	27	23,620	875	1411				
F	1938	34	10,190	300	308	*F* vs. *I*	163	109	N.S.
G	1938	32	4270	133	163	*G* vs. *F* *G* vs. *H* *G* vs. *I*	166 227 330	60 76 100	S. S. S.
H	1938	36	12,970	360	422	*H* vs. *I*	103	119	N.S.
I (Control)	1938	36	16,670	463	574				

TABLE X

Trichostrongylus spp. (INTESTINAL)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	14,590	486	387				
B	1937	24	12,860	536	538				
C	1937	27	13,310	493	597				
D	1937	29	15,140	522	489				
E	1937	27	12,970	480	599				
F	1938	34	20,150	593	581	*F* vs. *I*	604	332	N.S.
G	1938	32	10,680	334	415	*G* vs. *F* *G* vs. *H* *G* vs. *I*	259 788 863	124 227 325	S. S. S.
H	1938	36	40,390	1122	1286				
I (Control)	1938	36	43,100	1197	1898				

TABLE XI

NODULAR WORM (*Oesophagostomum columbianum*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	39	1.3	1.7	*A* vs. *C*	0.8	0.7	N.S.
B	1937	24	31	1.3	1.8				
C	1937	27	58	2.1	3.1				
D	1937	29	41	1.4	2.3				
E	1937	27	43	1.6	2.0				
F	1938	34	20	0.6	2.0	*F* vs. *I*	0.5	0.6	N.S.
G	1938	32	22	0.7	1.4				
H	1938	36	27	0.7	1.2				
I	1938	36	41	1.1	2.6				

the differences it was noted that one individual infection of 120 worms increased the standard deviation to such an extent that it was necessary to use the control group without this individual in the final estimation.

In the case of *Trichostrongylus* (including *T. axei*), tetrachlorethylene in capsules was shown to have some anthelmintic action; this was more definite against the intestinal species, as there was a highly significant reduced number in Group *G*.

Cooperia was affected by tetrachlorethylene capsules to a degree similar to that in *Trichostrongylus*.

Nematodirus was affected to some degree by the tetrachlorethylene emulsion.

Experimental Treatment for the Prevention of Nodular Worm Infection

In parallel with the tests of 1938, a small scale experiment was conducted in an attempt to demonstrate prevention of nodular worm infection. Twelve grade Shropshire ewes each with a single lamb that had not been out of the sheep barns were available. Six of these were chosen at random for a treatment designed to remove the adult nodular worms from the colons and thus prevent contamination of the pasture on which they were to graze. It was at this time apparent that the free-living stages of *Oesophagostomum* did not survive the winter months on pasture in Eastern Canada (13) thus the necessity of developing an efficient method of removing the source of infection (i.e. the adult worms in adult animals) was clear.

The method described by Ross and Gordon (9) of using enemata of solutions of sodium arsenite was tested. A brass injection pump that delivered 4 fl. oz. at each stroke was designed; a tube connected the pump to a source of supply and another tube, 20 in. long, was connected to the nozzle for insertion into the rectum and lower colon. This apparatus was tested on four sheep that were slaughtered immediately after the injection and it was found that a suspension of carbon particles could be easily introduced into the caecum of these animals, well past the normal habitat of *Oesophagostomum*.

On May 4, 1938, the six ewes were given preliminary enemata of tepid water, to remove excess faecal material. After a 30 min. interval, 1 qt. (40 fl. oz.) of water containing 0.25 gm. sodium arsenite was slowly injected into the colons, the hindquarters of the animal being held up for $2\frac{1}{2}$ min. The animals were kept on a bare lot for 24 hr., then turned on to a pasture of 2 ac. The control group was turned on to an adjoining pasture of equal size.

On June 3, 1938, the treated group received a drench of the tetrachlorethylene emulsion, preceded by 5 cc. of 5% copper sulphate solution. The ewes received 7.5 cc. tetrachlorethylene and the lambs 2.25 cc. tetrachlorethylene. The control group received tetrachlorethylene in capsules, the ewes 5 cc., and the lambs 2.5 cc.

At this time the egg counts in the ewes ranged from 400 to 1600 eggs per gram, there being no difference between groups.

The same tetrachlorethylene treatments were repeated on July 21, 1938.

One lamb from the control group had died of pneumonia on July 20, 1938; autopsy revealed two specimens of *Haemonchus*, no *Oesophagostomum*, but six nodules; the other worms found were very few in number.

On September 15, 1938, the ewes were removed and the lambs were again treated with the tetrachlorethylene preparations, as above.

The lambs were slaughtered on November 7, 1938. Autopsies revealed no significant difference in numbers of parasites of any important species. The treated group had an average infection of 3.2 adult nodular worms and 302 nodular lesions; the untreated group had an average infection of 3.0 worms and 101 lesions.

This treatment was unsuccessful in preventing the occurrence of nodular disease in lambs and did not reveal any advantage of an emulsion of tetrachlorethylene over that drug in capsules for the removal of other worm infections.

Observation on Worm Infections on Test Plots

The Central Experimental Farm conducts annual tests to determine the value of plots subjected to various fertilizing practices and botanical observations. Sheep and steers are used as the criteria of nutritive value. Of necessity these plots are heavily grazed and thus the factor of parasitic disease in lambs could affect the results to an important extent. Until recently the ewes and lambs were subjected to fairly frequent treatments throughout the grazing season, in order to prevent losses from verminous gastritis; in 1937 the Cunic drench was used. On October 1st of that year the 41 lambs remaining on the experimental plots were slaughtered and the viscera were examined in detail for evidence of parasitic disease.

The routine treatments had, apparently, kept *Haemonchus* under reasonable control. The high incidence of intestinal damage due to lesions of oesophagostomiasis appeared to be a major factor in the unthriftiness of some of the lambs from these pastures. It is apparent, however, that some individuals were able to thrive even though a considerable number of nodular lesions were present in the intestines. An analysis of apparent differences in growth rates shows that the 29 lambs that had not more than 200 nodules had an average daily gain of 0.30 lb. ($S.D.$ = 0.104) during the last 30 days of life, whereas the 12 lambs having over 200 nodules had an average daily gain of 0.22 lb. ($S.D.$ = 0.113). The apparent difference of 0.08 lb. is significant. There was no significant difference between the gains of males and females (means 0.282 and 0.289, respectively) and no correlation between birth weights and final gains. The 31 lambs that gained 0.2 lb. or more per day during September had an average of 118.8 nodules ($S.D.$ = 85.7) and the 10 that gained less than 0.2 lb. had an average of 269.3 nodules ($S.D.$ = 181.4); the difference is significant.

The same grouping showed no significant differences between the numbers of other worm species (Table XII). Some individual infections were of

TABLE XII

AVERAGE HELMINTH INFECTIONS IN LAMBS GROUPED ACCORDING TO THRIFTINESS DURING THE LAST 30 DAYS OF LIFE (SEPTEMBER), 1937

	Haemonchus	*Ostertagia*	*Trich. axei*	*Moniezia*	*Monodontus*	*Nematodirus*	*Cooperia*	*Trichostrongylus*	*Trichuris*	*Chabertia*	*Oesophagostomum*	*Nodules*
Group *A*												
Av.	69	917	565	23.9	65.4	918	1150	1345	29.3	9.3	4.6	118.8
S.D.	97	1097	305	81.2	67.4	1780	310	1193	34.6	7.2	2.7	85.7
Group *B*												
Av.	52	897	788	6.6	52.5	878	2860	5773	57.8	24.0	7.5	269.3
S.D.	73	1150	613	15.2	56.8	1795	5015	7701	52.1	25.4	9.4	181.4

Group A is 31 lambs which gained 0.2 lb. or more per day during September on pasture.
Group B is 10 lambs which gained less than 0.2 lb. per day during September on pasture.

interest; two lambs carried 298 and 362 tapeworms (*Moniezia expansa*), respectively, and weighed 78 and 85 lb. at slaughter with an average daily gain for September of 0.33 and 0.35 lb. The number of *Haemonchus* found was 423 in one animal (the only one with more than 250); it had a rate of gain of 0.42 lb. One heavy infection of *Trichostrongylus* occurred (29,000), but this was complicated by 542 nodules on the intestines and by 17,000 Cooperia worms; this lamb gained only 0.03 lb. per day during September. The maximum number of adult *Oesophagostomum* found was 25, these being present in the lamb with 542 nodules. Hookworms (*Monodontus trigonocephalus*) were present in all animals and ranged from 4 to 283 in numbers; the difference in growth rate between five lambs with more than 100 hookworms and the rest which had less than 100, was insignificant. The maximum numbers of *Ostertagia* and *Nematodirus* found were 5500 and 8500 respectively; these moderate infections occurred in thrifty lambs (average daily gains, 0.34 and 0.35 lb.).

Discussion of Preliminary Observations of 1937-38

The experiment in medication conducted during 1937 and 1938, and the observations made in regard to the actual problems of parasitic disease on the Central Experimental Farm pastures, tended to show that the unsolved problem of control of oesophagostomiasis was the most urgent field for further work.

It was apparent that *Haemonchus* could be kept under control by routine medication of the flock and that any one of several anthelmintics could be used for the purpose. In view of parallel work on the probable destruction of all or most of the free-living stages of *Haemonchus* on the pastures by the winter conditions (13), the treatment of the flock before they went to pasture probably accounted for the relative freedom from heavy infections in the lambs in the untreated group in 1938.

An alternation of treatments at intervals with tetrachlorethylene and Cunic appeared at this time to be indicated in the event of harmful *Trichostrongylus* infections.

It was apparent that not one of the methods of medication tested was reliable for removing tapeworms from lambs. However, as observations had continually failed to show a definite pathogenic effect from these parasites, in these flocks and others, continued work on control measures did not seem to be justified.

The finding that the emulsion of tetrachlorethylene was not more effective than this drug in capsules was unexpected. However, it should be noted that Gordon and Whitten (3) tested the emulsion in 1940 and found that it attained up to 98% efficiency in reducing the egg output of *Trichostrongylus* and *Ostertagia*. These results indicate that their method of administration, involving prior stimulation of the reflex closure of the oesophageal groove by copper sulphate solution, is superior to the one used in these tests.

The failure to show value in the mixture of copper arsenate, copper tartrate, and calcium hydroxide does not detract from the work of Mönnig (7) because his method of administration was modified and, also, the numbers of adult *Oesophagostomum* present in the lambs used were comparatively small. The writers did show, however, that the modification is of less value as a general anthelmintic for use in eastern Canada than other methods tested at this time.

In the smaller scale test of the enema treatment of ewes for the removal of *Oesophagostomum*, the results indicated that either the adult worms were not effectively destroyed or the infective stages were present on the small pastures, having survived the winter of 1937–1938. In light of more recent results it is now known that this treatment of the adult sheep was inefficient, and that the lambs acquired the infection as a result of contamination of the pastures by the ewes during the season of the experiment.

The Situation in 1939

No tests were made with the flocks of the Central Experimental Farm during this year. The routine of anthelmintic medication of breeding stock before the pasture season and the treatment of lambs at approximately 40-day intervals throughout the summer, was adopted, with due regard to the findings of 1937–38. In order to give practical application to the findings a bulletin on control of stomach worms for the use of sheep owners in eastern Canada was issued (10). Although no detailed examinations of the intestinal tracts of the 1939 lambs from this flock were made, casual observations of the viscera at the time of slaughter indicated that lesions of oesophagostomiasis were more numerous than in previous years. This might indicate that *Oesophagostomum* was becoming more heavily established in ewes that had come in 1936 from the Prairie Provinces where this worm parasite does not occur.

The lambs were marketed in the months shown in Table XIII.

TABLE XIII

ANIMALS MARKETED FROM C.E.F. FLOCKS, 1937–41, FOR SLAUGHTER AS "FINISHED" LAMBS

Year of birth	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Total No.
1937					57	47	121	26	17	8	18	7	301
1938				27	38	55	16	58	—	11	84		289
1939				33	44	53	60	56	31	33			310
1940				40	28	35	76		65	32	53	23	352
1941	4	13	58	40	—	43	49	38	20	28			293

In the meantime, independent work was conducted at the Institute of Parasitology in an effort to determine means of effectively removing adult *Oesophagostomum* from breeding stock during the winter or early spring months and thus protecting pasture lands from contamination with the free-living stages of this worm.

Further Tests in 1940

Following the demonstration in 1939 of phenothiazine as an anthelmintic for sheep by Harwood *et al.* (6), Swales (11), Roberts (8), and Gordon (2), and the development of a practical method of using this chemical as a highly effective means of removing *Oesophagostomum* and other worm parasites, further tests were arranged. The same flocks as used in 1937 and 1938 at the Central Experimental Farm were made available for the tests, as well as other smaller flocks of pure bred animals. Lambing was completed towards the end of April. Between this time and the first week in May all the adult sheep were dosed with approximately 40 gm. of phenothiazine in the form of tablets as previously described (11 and 14). The dose was given without prior fasting and the method was found to be practical for use in relatively large flocks; four compound tablets were administered to each of approximately 70 sheep per hour.

Following the treatment the flocks were retained in barns and enclosed for a minimum of four days, after which they were turned to the permanent pastures for the season.

The first lot of lambs was marketed for slaughter in July and even though this month had been shown to be the time of optimum numbers of *Haemonchus*, very few of these parasites were found in the sample lambs examined in detail. The relative freedom from this pathogenic stomach worm led to the determination that no further treatment of the adult stock and no treatment of the lambs would be administered in 1940. Two hundred and eighteen lambs

from these flocks were examined at slaughter in lots 40, 28, 35, 71, and 44 in July, August, September, October, and December, respectively. The intestines were examined for the presence of lesions of oesophagostomiasis and all such nodules were counted. Detailed examinations for intestinal worms for all species were made on 4, 4, 4, 6, and 44 lambs in the above lots. The results are shown in Table XIV.

TABLE XIV

COMPARISON OF INFECTIONS IN UNTREATED LAMB GROUPS

Year	No. lambs	Stomach worms					
		Haemonchus		*Ostertagia*		*Trichostrongylus*	
		Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*
1938	36	54.3	69.3	1979	3216	234	207
1940**	22	13.0	17.7	594	552	478	1004
1941	13	3.8	6.0	505	394	52	69

		Small intestinal worms											
		Moniezia		*Monodontus*		*Nematodirus*		*Cooperia*		*Trichostrongylus*		*Strongyloides*	
		Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*
1938	36	11.3	15.1	9.2	117.6	2457	4302	463	574	1197	1898	229	205
1940**	24	29.5	45.3	8.5	11.5	1030	1932	606	848	1515	2519	194	199
1941	13	12.2	12.9	0.6	0.9	1048	1190	65	66	1455	1526	214	127

		Large intestinal worms					
		Trichuris		*Chabertia*		*Oesophagostomum*	
		Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*
1938	36	16.9	26.6	2.8	4.8	1.1	2.6
1940**	24	23.0	23.1	12.0	13.8	0.3	0.7
1941	13	24.0	27.5	6.0	11.4	0	0

			Nodule lesions*		Nodule lesions (Autumn months only)		
			Av.	*S.D.*	Av.	*S.D.*	Reduction, %
1938	138	87***	49.4	59.0	72.6	64.1	0
1940	218	115***	5.9	10.6	9.7	13.1	86.5
1941	84	34***	0.26	0.94	0.65	1.39	99.1

* *This includes the treated and untreated lambs examined in 1938.*

** *Six animals, picked at random from the December lot of 44, are included, in order to make them comparable with numbers from other months.*

*** *Autumn months only.*

The improvement in condition of the flocks was marked, and, in spite of the fact that pastures were sparse in the autumn due to the use of part of the ranges for other purposes, no condition that called for anthelmintic medication was encountered. Only 16 lambs out of 218 examined had more than 20 nodules but 133, or 61%, had one or more nodules on the intestines; this is compared with an average of 49.4 nodules in the lambs killed in 1938, at which time 90% had some nodules. Figures for lambs slaughtered after July in 1938 show that 100% had nodules on the intestines; comparable figures for 1940 show an incidence of 75.7%.

The 86.5% reduction in numbers of nodules in the lambs slaughtered in the autumn of 1940, when compared with those in 1938, indicated that the system of preventive treatments was well founded.

The Completion of the Tests in 1941

The great reduction in nodules in the lambs of 1940 due, obviously, to the removal of the majority of the nodular worms from the adult sheep, and the fact that very few *Oesophagostomum* could be found in the lambs, suggested that a further treatment of the breeding stock in 1941 might eliminate this parasite from the flocks in question. Accordingly, all the adult sheep and the yearlings carried over for breeding stock were treated in April, 1941, after lambing was completed and at least one day before they were turned to pasture. A dose of 40 gm. of phenothiazine in the form of four Phenothiazine Tablets Merck was administered to each ewe and ram and 30 gm. to each of the yearlings.

The lamb crop of 1941 was very thrifty, the majority of the animals being sent for slaughter as market lambs (over 80 lb. in live weight) before the end of the summer. Samples were examined in June, October, and December. In the last two lots marketed (October and December) when nodules should be present in greatest numbers, only 10 out of 34 lambs had any lesions and only one animal had more than three.

That other worm parasites did not become numerous in spite of no anthelmintic medication of the lambs is of more than casual interest. The significant decrease in *Cooperia* indicates that the free-living stages of this parasite did not survive the winter months on pasture and that a high percentage of the adult worms were removed from the flock by the treatment with phenothiazine. The significant decreases to a very low level of *Haemonchus* and *Monodontus* was more to be expected, as winter survival in eastern Canada of their eggs or larvae has not been demonstrated; also, phenothiazine is highly effective against the adult parasites. Nevertheless, this demonstration of control of the highly pathogenic *Haemonchus* on permanent pasture without recourse to medication of lambs is highly encouraging.

It is apparent that *Oesophagostomum* has been reduced to a level too low for survival and that even without anthelmintic medication in 1942 this important parasite would not occur in the lambs of these flocks on the Central

Experimental Farm pastures. This hypothesis is founded on the improbability of even one pair of *Oesophagostomum* remaining or occurring in one animal when the greatest number of nodules formed in one lamb in 1941 was seven. In this flock the proportion of adult worms to nodules approximated 1 : 40 during 1937 and 1938, and only seven lambs out of 44 examined in December, 1940, harboured both sexes of *Oesophagostomum*.

During the experiments of 1941 the process of dosing was carried out without difficulty and no animal was injured by the treatment.

Acknowledgments

Grateful acknowledgment is made to Canada Packers, Ltd., Hull, Que., for their continued help in this work. This company undertook to cool, pack, and ship to the Institute laboratory some 400 sets of intestines during the four years of work, and all of them were received in excellent condition. In this connection particular thanks are due to Mr. W. J. Hodder, Superintendent, Mr. H. Knox, Chief Buyer, and Dr. A. C. Tanner, Inspector-in-Charge, Department of Agriculture. The authors gratefully acknowledge the continued interest and encouragement of Mr. G. W. Muir, Dominion Animal Husbandman, and the essential facilities he placed at their disposal.

They also wish to acknowledge the help and encouragement given by Dr. T. W. M. Cameron, Director, Institute of Parasitology, Dr. J. M. Swaine, Director, Science Service, Department of Agriculture, Dr. E. A. Watson, Dominion Animal Pathologist, and the late Mr. A. A. MacMillan, Associate Chief, Production Service.

References

1. CURTICE, C. U.S. Dept. Agr. Bur. Animal Ind. Bull. 1890.
2. GORDON, H. McL. Australian Vet. J. 15(6) : 245-252. 1939.
3. GORDON, H. McL. and WHITTEN, L. K. J. Council Sci. Ind. Research, 13(2) : 81-85. 1940.
4. HALL, M. C. and FOSTER, W. D. J. Agr. Research, 12(7) : 397-447. 1918.
5. HALL, M. C. and SHILLINGER, J. E. Am. J. Trop. Med. 5(3) : 229-237. 1925.
6. HARWOOD, P. D., HABERMANN, R. T., and JERSTAD, A. C. Vet. Med. 34(7) : 440-443. 1939.
7. MÖNNIG, H. O. Onderstepoort J. Vet. Sci. Animal Ind. 5(2) : 419-438. 1935.
8. ROBERTS, F. H. S. Australian Vet. J. 15(6) : 237-244. 1939.
9. ROSS, I. C. and GORDON, H. McL. The internal parasites and parasitic diseases of sheep. Angus and Robertson, Ltd., Sydney, Australia. 1936.
10. SWALES, W. E. Can. Dept. Agr. Pub. 639. 1939.
11. SWALES, W. E. Can. J. Comp. Med. 3(7) : 188-194. 1939.
12. SWALES, W. E. Can. J. Research, D, 18(1) : 29-48. 1940.
13. SWALES, W. E. Can. J. Comp. Med. 4(6) : 155-161. 1940.
14. SWALES, W. E. Can. J. Research, D, 18(7) : 266-271. 1940.
15. SWALES, W. E. Agr. Supplies Board, War Time Production Ser. Special Pamphlet 51. 1941.

29 OCT 1942

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. JUNE, 1942 NUMBER 6

STUDIES OF WATERFOWL IN BRITISH COLUMBIA

BUFFLE-HEAD[1]

BY J. A. MUNRO[2]

Abstract

Charitonetta albeola is a common summer visitant to parts of the interior of British Columbia and is abundant on the coast region in winter. Adults arrive in the interior in advance of the yearlings and the sex ratio in early spring flocks is predominantly male. Later, both age groups associate for a short time, then the paired adults become established on nesting territories. Most of the yearling males, and subsequently the adult males, disappear for six weeks or longer. They eclipse and begin to appear again on certain lakes in August but are not plentiful until late September or early October. The yearling females remain on the breeding grounds in flocks throughout the summer. Courtship which is observed first on the coast in March reaches its greatest intensity on the interior lakes in April. Males vigorously defend their nesting territories. Eggs are laid in May and by the last week of June most of the young have appeared. Breeding females leave their broods in order to moult before the young have reached the flying stage and associate with flocks of yearling females that have gathered on certain waters where food is abundant. At this time all are excessively wary. In the interior aquatic insects are the chief food of downy young, adolescents, and adults. On certain lakes amphipods and molluscs are important foods. Fishes, where available, form a minor part of the diet. Seeds of aquatic plants are the main vegetable food except on Okanagan Lake where a larger amount of other plant material is consumed. On salt water, crustaceans and molluscs are first and small fishes, second in importance. The buffle-head is highly regarded as an object of beauty. In the interior it is of economic value as food but is not held in esteem on coast waters. No evidence of it eating commercially valuable fishes was obtained and its consumption of salmon eggs, noted on coast streams, does not reach significant proportions.

Introduction

The buffle-head, *Charitonetta albeola* (Linn.), may be described as a sea duck which undertakes a yearly migration into the interior to nest. In winter it is comparatively abundant on all sheltered coast waters where its tameness, and the handsome appearance of the adult males, make it a general favourite with the observing public; so also in spring their intense sexual activity renders them more than ever conspicuous.

The purpose of this paper is to record the distribution, numerical status, and seasonal movements of the species in British Columbia, as well as the plumage sequence, behaviour, and the food as determined through the study of stomach contents. General observations have been made in this province since 1911 but the greater part of the material presented was obtained in the Cariboo region during the years 1936 to 1941 inclusive.

[1] *Manuscript received February 16, 1942.*

Contribution from the National Parks Bureau, Department of Mines and Resources, British Columbia, Canada.

[2] *Chief Federal Migratory Bird Officer for British Columbia.*

It is of interest to note the various similarities between the buffle-head and Barrow's golden-eye, *Glaucionetta islandica* (Gmelin) (6). Their summer distribution in British Columbia, both geographically and in respect to habitat, is identical; so also in place and time are their migratory movements. Courtship behaviour follows the same general pattern; both are tree-nesting ducks and the downy young of the two species are closely similar. The same sequence of moults is followed, their seasonal movements, the local distribution of different age groups and general behaviour on the nesting grounds are much the same.

Distribution, Seasonal Movements, Summer Populations

Summer

The buffle-head nests in the interior of the province east of the Cascade Mountains, the centre of abundance being the dry belt region comprising the Nicola, Kamloops, Cariboo, and Chilcotin districts. It is less abundant in the Okanagan, reported as common on the Arrow Lakes (4) and scarce in the East Kootenay (W. B. Johnstone, personal letter). It nests at Ootsa Lake (J. L. Shelford, personal letter) and is recorded as breeding at Hazelton (7) and in the Peace River district (2). It is not recorded from the Skeena region (10) nor from the Stikine region (9) but is a regular and fairly abundant migrant at Atlin (11).

Spring Migration

A migration from the south and from the coast takes place in March and April. Known migration routes are the Columbia and Okanagan Valleys, the Fraser and Thompson river system, the Harrison, Lilloet, and Seton lake system and the Cheakamus river and lake system. Undoubtedly many other rivers farther north are fly ways into the interior.

In the Okanagan and Nicola regions the spring migration reaches its peak in the latter part of April or early May when on favourable lakes of small size the aggregate of birds (that seldom are together in one flock) number 25 to 50. The following counts represent maximum numbers seen.

Falcon Lake	—	April 14, 1940: 40.
Munson's Lake	—	April 19, 1934: 53; May 8, 1937: 47.
Trapp Lake	—	April 19, 1933: 33.
Rawlings Lake	—	April 24, 1937: 25; April 12, 1940: 20.
Tule Lake	—	April 25, 1934: 40.
Swan Lake	—	May 11, 1933: 57.

Most of the birds in these flocks are transients and all except a relatively small number move out before the end of May.

Farther north in a more thickly populated nesting region concentrations have been observed on 103 Mile Lake where exceptionally favourable food

conditions exist, but the average spring flocks are of similar size to those seen elsewhere.

103 Mile Lake	—	April 16, 1941: 250.
105 Mile Lake	—	April 16, 1941: 50.
Cummings Lake	—	April 18, 1941: 56.
Mirage Lake	—	May 11, 1939: 37.
Beaver Dam Lake	—	May 10, 1939: 40.

There is still at this time a considerable number in small flocks moving up the coast; in April this movement includes both adults and yearlings usually in separate flocks. Adults migrate inland in advance of the yearlings and by the middle of May the latter predominate in the coast flocks. This was the case at Massett, Queen Charlotte Islands, May 1 to 20, 1920, where daily observation of the population revealed a steady reduction in the number of adults as the month advanced, so also at Tlell, Queen Charlotte Islands, where on May 4, 1935, the population consisted of three adult males and 22 females, which were considered to be yearlings. The latest recorded date of spring migrants at Port Hardy, Vancouver Island, is May 8, 1940 (Allan Lyon, personal letter), at Tlell, May 14, 1935, and at Massett, May 20, 1920.

Segregation of the two age groups has been observed early in the spring, as at Hammond Bay, Vancouver Island, March 28, 1941, where a flock of 37 adult males and 32 females, also probably adults, contained no yearling males. That the two age groups separate while on the coast and migrate inland at different times is also suggested by observation of spring flocks in the interior. Thus of more than 500 examined in the Kamloops and Cariboo regions April 14 to 18, 1941, all the males and, so far as could be determined, all the females were adults. One yearling male was seen April 23 and two on April 24; a month later yearling females were plentiful and yearling males were seen daily.

Summer Populations

During late April and early May an association of adults and yearlings on the nesting grounds is general. The membership of these associations is constantly changing; adults pair off and move elsewhere, so also the yearling males, now rapidly becoming lighter in colour and more conspicuous, gradually disappear. Later the numbers of a flock may be temporarily increased by the arrival of males that have fulfilled their part in the reproductive process. At the same time other males, probably mated later, are still conspicuous in appearance and behaviour on their nesting territories. Thus at 103 Mile Lake, June 3, 1937, 15 adult males dived for food, rested on the water or dressed their plumage in company with 30 yearling females, while the same day on adjacent 105 Mile Lake mated males were actively displaying, rushing at each other across the water, and making nuptial flights around the lake. Other dates for the early flocking of postbreeding males on the nesting ground are: Westwick Lake, June 3, 1937, 30 adult males in a total population of 50; Hunter's Lake, south of Kamloops, June 11, 1938, 17 males in company with

five faded yearling females. The latest dates for the appearance of postbreeding males prior to the summer moult are: July 3, 1938, when 11 on 103 Mile Lake and six on 105 Mile Lake were counted, and July 11, 1938, when a single male was seen.

Following the brief stay of the postbreeding males with the yearling females, there may be a short period when the rafted population is composed exclusively of the latter age group. Some associate in small bands up to 15 or 20; others, perhaps the greater number, assemble on certain lakes, not always those which spring flocks have occupied, and remain there until after the moult in August. These serve as nuclei to which are attracted first the local breeding females and then adolescent young, then postbreeding females that have abandoned their broods on other lakes of the region and finally, towards the end of the summer, moulting adult and yearling males that have renewed their flight feathers.

The most notable concentrations of non-breeding females, with later additions of adults and young, were observed at 103 Mile Lake. The physical characters of this lake and the behaviour of the rafted ducks as observed in 1936 have been described elsewhere (5). Subsequent observations are briefly summarized as follows.

On June 2, 1937, 40 individuals of which 15 were adult males and the remainder yearling females were together in one flock. On August 4, the population had increased to 500 (estimated), of which most were considered to be non-breeding yearling females. Some were flightless but the majority took wing at 150 yards or at an even greater distance when approached by a canoe. A number of flightless birds examined at close range through binoculars were faded to light drab on head and flanks. This association had almost completely dispersed by August 25 when only 20 were on the lake; these were chiefly young that had not attained full power of flight.

The population on July 3, 1938, consisted of 10 adult males and 11 yearling females and on July 26, of approximately 130 females and one yearling male. The latter was losing its flight feathers and able to fly only a short distance. On August 16 the number was reduced to 40, most or all being yearling females.

No concentration took place in 1939, and on July 18, the population consisted of two adult females with a total of eight young.

On June 12, 1940, a total of 12, of which eight were adult males, was present; these were associated with Barrow's golden-eye. On August 6, approximately 250, all females, comprised the largest proportion of a raft of diving ducks which otherwise was composed chiefly of Barrow's golden-eye and lesser scaup ducks, *Nyroca affinis* (Eyton). When disturbed, as they were half a dozen times, the buffle-heads rose in small bands and after circling once or twice, left the lake.

Other summer concentrations have been observed, for example: 64 at Mirage Lake, August 4, 1938; 75 at Tunkwa Lake, August 9, 1939; 300 at Minnie Lake, August 10, 1939.

At Rush Lake, Springhouse, Cariboo, between June 13 and July 10, 1941, the number of postbreeding males and second year males and females changed from day to day. The adult males were in full breeding dress, the second year males in various stages of moult with varying amounts of white on face and back. Some rose heavily from the water but all were strong on the wing and exceedingly difficult to approach. For several days one of the yearling males accompanied a female with brood of downy young. The changes in this population are shown in the following counts.

	Adult ♂	Yearling ♂	Yearling ♀
June 13	3	5	4
June 14	0	3	2
June 15	5	7	0
June 18	4	6	2
July 7	0	1	6
July 10	0	0	12

A female with brood of five, about one-quarter grown, were present on June 13. Other females with broods of downy young as indicated appeared on the following dates, 11 on June 20, 6 on June 23, 4 on June 24, 8 on July 7.

The following are examples of typical summer populations on small breeding lakes.

On Tatton Lake, June 11, 1940, the population consisted of three mated pairs, three females with broods of downy young numbering 5, 6, 7, and 12 yearlings of which 10 were females. On August 3, 1940, the total present was 50 (estimated) consisting of adult and yearling females and young of the year.

On 105 Mile Lake, June 12, 1940, the population was three mated pairs and one female with brood of eight downy young. On August 5, 1940, a total of 40, most of which were associated with a raft of lesser scaup duck, included full grown young, adult and yearling females, and three adult or yearling males in partial eclipse.

On the nesting grounds adult and yearling males are scarce or absent for approximately six weeks from early July to mid-August. In 1936, none was seen and in 1937 five were recorded as follows: two yearlings, Disputed Lake, July 25, in company with two yearling females; two probably adult males, Green Lake, July 27, one completely flightless, the other capable of fluttering over but not rising from the water; one flightless bird in partial eclipse, 103 Mile Lake, August 4. In 1938, one partially moulted yearling male, 103 Mile Lake, July 26, was the only record; subsequently none was seen until late August. No males were recorded in the summer of 1939 until August 22 when the first of those that had renewed their flight feathers appeared on 103 Mile Lake.

In the summer of 1940 adult and yearling males were more conspicuous on the nesting grounds than had before been observed. The following are definite records:

105 Mile Lake, Aug. 5: 3; 148 Mile Slough, Aug. 9: 1;
Cummings Lake, Aug. 10: 1; Watson Lake, Aug. 12: 1;
103 Mile Lake, Aug. 14: 2; Tatton Lake, Aug. 14: 1.

That this general absence of males from the nesting grounds covers roughly the period of moult and that the period is short, a month to six weeks, suggests that they retreat to waters not far distant. It is suggested that eventually important summer concentrations of males will be found on some of the ornithologically unexplored lakes in central British Columbia. Very likely they will be in large flocks as are most of the yearling females at this time and, judging from the actions of those few summer males that have been encountered, they will be exceedingly wary.

Autumn Migration

Small numbers of moulted adult and yearling males reappear on the nesting grounds in late August but in some years they are uncommon, even as late as the last week in September. Thus at Longbow Lake, September 23, 1940, only three were detected amongst three flocks totalling 150 and at Irish Lake, September 25, 1940, a flock of 50 contained only one. A total of 22, 103 Mile Lake, August 20, 1937, and five, 105 Mile Lake, August 22, 1939, represented exceptionally early appearances.

Adult males usually do not appear in numbers until October and at no time do flocks on the interior lakes contain the same percentage of adult males as do winter flocks on the coast. It seems likely that an early migration of males from the interior to the sea occurs in advance of the main migration, as in the case of the lesser scaup duck.

A limited southern movement from the nesting grounds in the Cariboo occurs in September but not until the first freeze-up, which usually takes place in late October, is there a general exodus. Following this the numbers on the lakes in the southern interior are increased by the arrival of these migrants. Later, as winter approaches in these lower latitudes, the bulk of this transient population moves south or west to the coast. A series of counts on Swan Lake, Okanagan, illustrates the rise and decline in numbers at this time:

1933 — Nov. 1: 100; Nov. 4: 200; Nov. 17: 50;
1939 — Sept. 30: 250; Oct. 28: 400; Nov. 18: 200.

Winter Population

Fewer buffle-head now winter on Okanagan Lake than 20 odd years ago. In the winter of 1915–16, 30 to 40 frequented the lake near Okanagan Landing even after a prolonged period of subzero weather (January 10 to 19) froze a large part of these waters; approximately the same number remained the following year. In the winter of 1917–18, five was the largest number seen in one day (January 26, 1918) and since that time it has been scarce or absent

on this lake during winter. No definite information is available concerning other possible winter populations in the interior.

The first autumn arrivals on the coast appear usually in September; a record of two females, Della Lake, Vancouver Island, August 20, 1923 (8) is considered unusual; the earliest recorded date at Port Hardy is October 5, 1939 (Allan Lyon). The numbers of this partially transient autumn population on the coast reaches its maximum in November. At this time small flocks are plentiful at many places and concentrations take place on particularly favourable feeding grounds; 200 at Esquimalt Lagoon, November 11, 1938, 80 at Shoal Harbour, November 12, 1938, 450 at Sooke Harbour, November 14, 1938, are examples.

Later in the autumn there appears to be a southerly movement so that the winter population in south coastal British Columbia is not as large nor as concentrated as it is during November. During the period December–February an average flock on southern Vancouver Island contains a dozen or so birds; the number of flocks, widely distributed on both salt water and fresh, and the total of individuals is greater than at first appears. For example the total of a number of flocks at Telegraph Bay, December 12, 1940, was approximately 150. It was the duck most frequently seen along the six miles of coast between Sooke Harbour and Tugwell Creek on December 11, 1940, and thereafter until March 17, 1941.

The population frequenting Departure Bay in winter varies from eight to 15 and when the herring begin to spawn in early March suddenly increases to 100 or more. Concentrations are apparent elsewhere at this time; 75 at Esquimalt Lagoon, February 25, 1934, 84 on March 28, 1938, 99 on March 17, 1939, 150 at Camp Slough, Chilliwack, March 29, 1935, are examples.

TABULATION OF SEASONAL MOVEMENTS

January–February	Association of all age groups on coast waters
March–April	Migration of adult population to interior
April–May	Migration of yearlings to interior, small flocks assemble on interior lakes; adults mate
June	Adult and yearling males disappear; young are brought to water
July	Populations restricted to adult females with young, yearling females and an occasional adult or yearling male
August–September	Concentrations of moulting adult and yearling females joined by flying young and by a few adult males and second year males now in adult plumage
September–October	All age groups assemble on suitable feeding grounds; commencement of migration
October–November	Migration south and to coast
November–December	Association of total population on coast waters

Reproduction

It seems probable that buffle-heads do not breed until their third year. The chief evidence for this is that large numbers of second year females associate in flocks on the breeding grounds at the time when breeding females are incubating or caring for young. Some inconclusive evidence suggesting exceptions to this rule follow.

A nesting female under daily observation on Boitano Lake, June, 1941, was conspicuously lighter in colour than normal breeding females, a condition indicative of yearling females at this season as explained elsewhere in this paper.

On a small mountain lake near Summerland, Okanagan, May 11, 1918, a nesting female was apparently mated to a male in second year plumage modified by extensive white areas on the face and the amount of white on flanks and shoulders characteristic of many yearlings in late April and May. The female joined this male each time she came to the water and in general the two acted as if mated; there were no other buffle-heads on the lake. Instances of yearling males associating in the nesting season with a particular female, in some cases where no other buffle-heads were present, also have been noted. For example on a pond near Vernon, June 8, 1928, and on Westwick Lake, Cariboo, June 17, 1941.

Courtship

Courtship display is first noticed on the coast in late January or early February and reaches its greatest intensity in the interior during April. At this time on the lakes where buffle-heads concentrate during the courtship period, the number of adult males usually exceeds the number of adult females; few second year birds are then present. At 103 Mile Lake, April 16, 1941, where the greater part of the local population was assembled, at least 175 of the total of 250 were adult males; some had paired, there were many trios (2 ♂, 1 ♀) and units of eight or 10 males accompanying two or three females. In one case eight males flew after a female and when the flock alighted four of these males rushed over the water towards the female. The males are exceedingly hostile toward one another. A mated male will swim under water toward an intruding male, emerging momentarily for direction if the distance separating them is considerable and then attempt to come up beneath the male on the surface so that it is obliged to rise. Should the second male alight again in the vicinity, the underwater attack is repeated. In one instance observed this performance was carried out by one male a dozen or more times, directed in each case against the same intruder. When the latter finally retreated the mated male swam toward the female, uttering a soft cooing note.

Courtship behaviour varies in detail and intensity; that of seven adult males, one yearling male, and two females was restricted to bobbing the head and a continuous "cooing" by several of the adult males. After five minutes

the flock dispersed and commenced feeding, four mated birds remaining together.

The behaviour on a small pond of four males that courted one female for 30 min. without interruption (April 22, 1941) was full of action and excited movement. Each of the males was in constant motion about the female; one that appeared to be her mate stopped displaying every few minutes and rushed over the water at one of the others. Four different actions were performed by the males.

1. A quick glide past or around the female, head bobbing rapidly.
2. A flight of a few yards, in which the pink feet were displayed; then a slide across the water, body partly upright and chest outthrust.
3. The male standing partly upright splashed the water with his wings, or, in a horizontal position, splashed alternately with wings and tail.
4. Bowing with vigorous forward thrusts that immersed the head.

All executed one or another of these actions simultaneously, the tempo rising to reach an excited turmoil which lasted for a minute or so and then subsiding to less sustained effort. The female swam quietly about the pond and to these demonstrations made no response. At another place the female of a pair danced in a partly upright position about the male; the latter did not respond.

Courtship actions on the water are interrupted by display flights conducted by a group of males, by single males, or by both sexes together. Thus at Rawlings Lake (April 12, 1940) five adult males and one female rose from near the centre of the lake, which is half a mile by one-quarter mile, and after circling most of its circumference in rapid flight, splashed into the water about 40 yd. from shore. In descending, the rose-coloured feet of the males were conspicuously displayed. Immediately after alighting all commenced to move across the water in a manner of progression that can best be described as a series of hops. In these movements the body was partly raised from the water and the wings half spread so that a continuous splashing took place. A single chuckling note was repeated in rapid succession, apparently by the males only. After several minutes in which this performance was continuous two other males joined the group and at once commenced to act in the same manner. Sometimes the single female in the group travelled ahead of one or more males and sometimes she followed them. Sometimes a male seized another male by the feathers on the back neck and held on while the lower male submerged as in copulation. These energetic and excited movements in which all took part continued for about five minutes, then all rose and alighted again about a hundred yards from shore where they began diving for food. Even while so engaged the urge to display was insistent and the males, upon rising to the surface, invariably started jerking their heads up and down in the courtship bow.

In the full tide of sexual excitement the awareness of the male for the female is dominant, greater than their reaction to danger. Thus at Swan Lake

(May 2, 1936) the author was paddling toward a trio of two males and one female which were swimming close together along the edge of the rushes. When the canoe was 70 yd. distant, the two males rose but the female continued swimming. A few moments later one of the males, after flying a quarter mile up the lake, turned around and headed straight toward the female. When 20 yd. or so from the canoe, with rosy feet advanced and wings down-curved, he checked his speed and dropped to the water beside her. A moment later the female rose and the male quickly followed.

Second year males may exhibit some of the pugnacity so characteristic of the adult male. Thus at Okanagan Landing (May 4, 1919) a young male which accompanied two mated pairs repeatedly attacked one or another of the older males.

Courtship display continues during the laying period and terminates abruptly when the males leave their territories to associate with other males and non-breeding females. At this time the cessation of intense, almost constant sexual activity and the tolerance of one male for another is in marked contrast to earlier behaviour.

Nesting

The dry, semiopen Cariboo region supports the largest summer population. Here pairs are found in occupation of alkaline ponds, sloughs, and small lakes; they are less attracted to large lakes and to those situated at altitudes above 4000 ft. The high mountain ponds sometimes occupied by breeding Barrow's golden-eye are seldom used by the buffle-head.

A breeding pair establishes a definite territory which the male vigorously defends from encroachment by other males. The territory may be an entire pond or, on larger waters, an area along several hundred yards of shore line. In the latter part of May and in early June, males may be seen alone on these territories during the time each day when the female is on the nest. In some cases at least the male is aware of the location of the nesting tree and the whereabouts of the female during her absence from the water, as is suggested by an incident that took place on a small lake, May 15, 1916. A nesting tree was hit noisily with a stick several times and before the female appeared at the entrance a male rose from another part of the lake and splashed into the water beside the tree.

An incubating female does not readily leave the nest even after the nesting tree has been rapped several times. One has been seen to thrust her head and neck through the entrance and remain in that position for several minutes before flying out. It was observed on another occasion that a female after being flushed from her nest returned to it immediately when the observer had left its vicinity. While egg-laying continues the male constantly companions the female during the time she is on the water; he is still violently excited by the appearance of other males and the sexual manifestations described above continue.

By the time the females have brought the young to the water it is not usual to find males on the nesting lakes unless there has been considerable difference in time between the laying of individual birds. This perhaps was the case at Tatton Lake, June 11, 1940, when a male joined a female with brood of downy young and remained with her for some time. This was interpreted to be the sexual reaction of a male whose mate was in the egg-laying stage of reproduction.

So far as known nests are invariably in trees, more often in dead trees standing in or close to the water. For this reason nests of the buffle-head are easier to find than those of Barrow's golden-eye which may be a mile or more distant from the lake where the female will later lead her young. The buffle-head nests that have been studied were located either by keeping a breeding female under observation until she flew to her nesting tree or by detecting the presence of down at the entrance to the nest. Old nesting holes of flickers that have enlarged with the tree's decay supply the necessary requirements. Two sites found beside a small alkaline lake in the Okanagan are characteristic.

The first was in a dead Douglas fir 8 in. in diameter that had been broken off 30 ft. from the ground. It stood on the lake margin and leaned toward the water; the top was hollowed to a shell of bark. The entrance to the nest was four feet from the top of the tree and one foot above the bottom of the cavity. On May 22, 1915, this contained 10 partly incubated eggs insulated by a large amount of down. Beneath the down, mixed with the rubbish at the bottom of the cavity, were shell fragments of eggs laid in a previous year.

The second, which contained nine fresh eggs on May 15, 1916, was near the top of a 30 ft. yellow pine stub standing in a foot of water six feet from shore.

At Cummings Lake, June 9, 1941, a Douglas fir stub 10 ft. in height standing in three feet of water 20 ft. from shore contained a nest and six eggs. The entrance, of irregular shape and 4 in. wide at the widest point, was five feet above the water; the nest cavity measured 6 in. by 8 in. (Fig. 1). When a canoe touched the bottom of the stub the female flew out and alighted on the water 50 yd. away.

At Boitano Lake, June 10, 1941, the actions of a female indicated that a nest was in the vicinity. She swam in a nervous manner back and forth close to shore across a space of 30 yd. or so and at short intervals uttered a barely audible nasal quack. The location of the nest, which contained nine eggs, was soon determined by the finding of fresh down on the rough surface of a poplar stub a short distance from the lake (Fig. 2). The stub was 14 in. in diameter at the base and had been broken off 10 ft. above the ground; the lower portion had rotted so that it moved at a touch and ants had used it as a backing for an accumulation of fir needles and rubbish. The entrance to the nest measured 3 in. in diameter; the nest cavity, a flicker's excavation several years old, measured 8 in. across and extended 12 in. below the entrance; the wood at the entrance and the interior walls were solid. In order that a

person's hand and arm could be inserted it was necessary to enlarge the entrance by whittling the hard wood with a knife. The placing of a ladder against the tree, the enlarging of the entrance to the nest, and the taking of measurements and photographs occupied over half an hour; during all this time the duck remained on the nest.

The stub was visited on each of 11 successive days. Except once when the buffle-head left while the observers were approaching, and another time when she was absent, the procedure was to strike the stub several sharp blows in an endeavour to make her leave the nest. This did not succeed in its object but on three occasions she left precisely 10 min. after exploring fingers had touched her on the back and head. Each time she covered the eggs before leaving. On other days her presence was determined by touch and she did not leave while the observers were in the vicinity. With one exception these visits were made between 8.00 a.m. and 9.15 a.m.; the exception was at 4.00 p.m., this being the occasion she was not on the nest. On the four mornings she was seen leaving the nest she flew directly to the lake and remained there an hour or longer. As circumstances did not permit continual observation, it was not possible to determine whether she had regular hours for feeding.

The stub stood 100 ft. from the water near the edge of a fir and poplar wood. Nearby a rail fence extended into the water; the lower rail, 150 ft. in a direct line from the nest, was used as a resting place between periods of feeding. The feeding ground, limited to an area roughly 50 yd. by 100 yd. and no doubt approximating the area of the nesting territory, was directly in front of this point (Fig. 3).

When the stub was last visited at 2.30 p.m., June 23, all but two of the eggs had hatched; one contained a dead embryo, the other was cracked and the contents dry. The young were gone. The female, recognized by the faded pattern on her upper parts, and six young were seen the same day on Rush Lake which is about 200 yd. from the nearest point of Boitano Lake. The former is well stocked with food, the latter is a "soda" lake containing no vegetation and an animal population restricted chiefly to phyllopods and corixids.

In view of the few references in literature to the actions of nesting buffle-heads the following minutiae of behaviour as observed from a concealed position on the morning of June 12, 1941, are submitted.

8.20. Buffle-head flew from nest and alighted on water 60 yd. from shore where she commenced feeding. During the next hour she was visited by another female buffle-head, probably a yearling, and later by an adult male and four yearling females. At 9.20 when an observer visited the nesting stub, the duck moved in close to shore and swam back and forth quacking softly. After 9.45 she was kept under close observation through 6× binoculars until she returned to the nest at 11.19.

9.45 to 10.05. Remained on water directly in front of nest within an area of approximately 200 sq. yd. of which the inner boundary was about 30 yd.

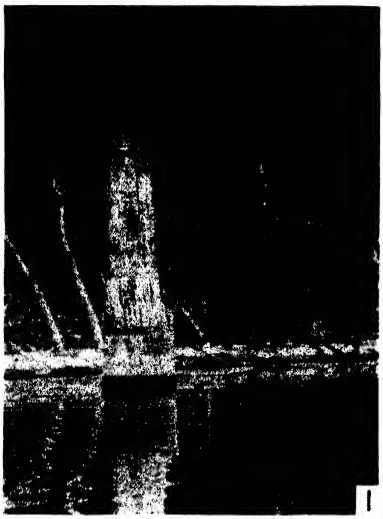
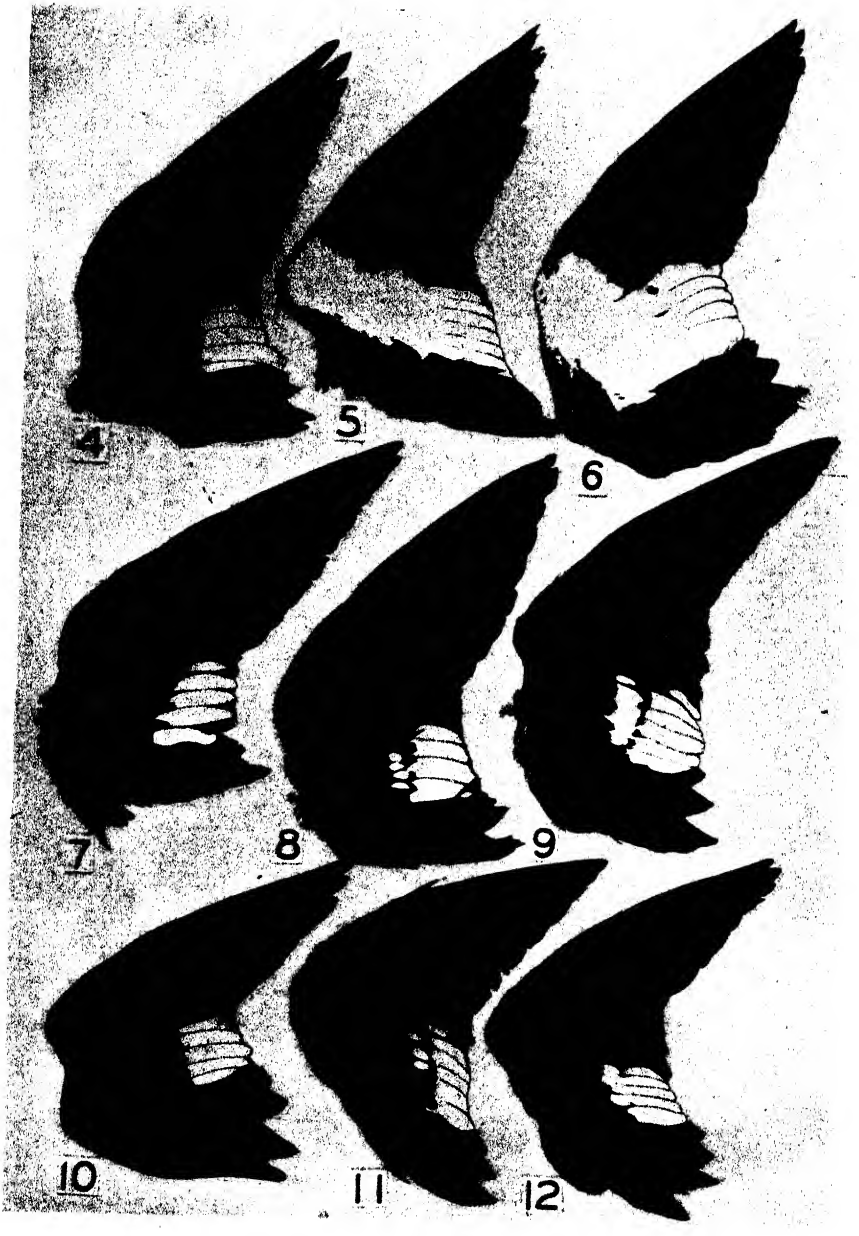


FIG. 1. *Douglas fir stub containing buffle-head nest, Cummings Lake, British Columbia.* FIG. 2. *Poplar stub containing buffle-head nest, Boitano Lake, British Columbia.* FIG. 3. *Nesting territory of buffle-head, Boitano Lake, British Columbia, nesting place in poplars at left, resting place on fence rail at right, feeding ground on lake at centre.*



FIGS. 4 TO 12. *Wing pattern of buffle-head, approximately ½ life size.* FIG. 4. ♂, *age approximately seven months.* FIG. 5. ♂, *age approximately 13 months.* FIG. 6. ♂, *adult.* FIGS. 6 TO 8. ♂, *immature, variation in pattern of greater wing coverts.* FIGS. 9 TO 12. ♀, *variation in pattern of greater wing coverts.*

from shore. Here she dived for food or floated on the surface sometimes dressing her plumage.

10.05. Swam to shore and climbed on to the lower rail of fence that slanted into water 10 ft. from the beach. She stood upright and kept shaking her tail vigorously, stretched out one wing then the other, ran her bill through the lower body plumage, the flank feathers, and under the partly opened wing. When not engaged in these movements she maintained an alert position, neck extended and head moving from side to side. At no time did she relax.

10.15. Turned sideways on rail and assumed horizontal position, then flew 75 yd. out on lake where she commenced feeding. She dived three times remaining under 14, 18, 18 sec. with intervals on the surface of 15 and 8 sec. Remained on surface 90 sec. preening plumage and shaking tail while in a horizontal position then standing partly upright and shaking wings. Two dives of 12, 13 sec. with interval of 15 sec. on surface; for 97 sec. she drifted motionless.

10.20. Flew to fence rail, climbed up and resumed dressing of plumage with constant vigorous tail shaking; alert as before.

10.27. Flew to feeding ground, submerged once then rose, made a circle over lake and alighted close to shore in front of nest site; remained there five seconds then flew to top of fence post on beach where she remained 35 sec. moving head from side to side.

10.30. Alighted on water again 20 yd. from shore; remained there 50 sec. then flew out on lake 75 yd. and commenced feeding. The length of time below the surface in four dives was 12, 15, 15, 17 sec., the length of time on the surface, 6, 6, 8 sec. After the final dive she stood up, shook her wings, and dressed her plumage. On the surface for 30 sec. She then rose, circled over the beach towards the nest, and returned to the water.

10.35. Flew towards nest site, returned to lake, and alighted near fence.

10.37. Climbed on fence rail and for 8 min. continued the activities described.

10.45. A female Barrow's golden-eye swam to fence rail; the buffle-head dropped to the water and swam out 50 yd., dressed plumage for a few seconds then flew straight towards nest site but turned and alighted on water.

10.47. Swam towards fence rail now occupied by female Barrow's golden-eye; swam in a circle about the other duck, drank several times; stood up and shook her wings, then flew to a position on the fence rail three feet from the golden-eye. Continued dressing plumage, stretching and shaking tail (all of which activities were also being practised by the golden-eye).

10.55. Flew to position 50 yd. from shore, rose immediately and flew towards nest, returned and alighted near shore.

10.57. Flew 40 yd. farther out and continued to feed for 3 min., then floated on the surface sometimes standing up to shake the wings.

11.04. Started to swim inshore towards fence rail.

11.07. Climbed to place on rail vacated by golden-eye; more tail-shaking and wing-stretching.

11.08. Slid off rail, flapped wings, and in 30 sec. climbed up again. For 11 min. she continued the activities described above but less vigorously and with longer intervals of alert watchfulness.

11.19. Flew directly from rail to nest.

Behaviour of Females and Young

The majority of the buffle-head broods appear on the water in June, the earliest record being June 12, 1940. The average number in a total of 58 broods, counted in July 1938 and July 1939, was seven. Females react to alarm in various ways; some are attentive parents and remain with their broods even after becoming excited by a threatened danger. For example, on Bridge Creek, July 13, 1937, when a female with eight half-grown young was overtaken on the narrow stream by a passing canoe she did not rise from the water nor increase her swimming speed beyond the ability of the young to keep up. When a family is isolated, as was the one mentioned above, brood formation under the care of a female is likely to be maintained until the young are well grown, but on well populated lakes females show less concern and if alarmed are likely to fly and leave the young to fend for themselves.

Thus on a small lake with a relatively large population broods tend to lose their identity while the young are still quite small. Under such conditions a thorough mixing of the population takes place; one female may have one or two young and another a dozen; one instance of a female with 18 young has been observed. In a population such as this some may leave their broods unattended and swim about in company on another part of the lake. The uncared for young may join other broods, remain together as a brood, or dissolve into several units. This condition has been noticed particularly on lakes where the surface is covered with lily pads or a heavy growth of *Potamogeton* or other similar plants. By reason of such obstruction the small young are not always able to keep up with the female, they are left behind and eventually become lost. Single young thus abandoned may wander for some distance and even leave the water as did a young bird about a week old which was captured (July 20, 1937) beside a road through thick woods one mile from the nearest water. When adolescent young are attracted to a raft of non-breeding females, they cease to maintain the brood formation and become individual members of the larger association.

In some cases the scattered, half-grown young, or even compact broods, are deserted permanently. Thus at Lily Pad Lake, August 7, 1937, only three broods of 5, 7, and 8 were accompanied by females while 2, 4, 5, 5, 6, and 10 young were unattended. The same condition was observed there on July 21, 1938, when four females accompanied broods of 1, 2, 3, and 5, and a total of 19 young, i.e., 1, 1, 1, 2, 3, 3, 4, 4 were not accompanied.

Broods sometimes are accompanied by two females: for example, at Buffalo Lake, August 7, 1937, this was so in the case of a brood numbering seven. As with Barrow's golden-eye (6), the second female usually is a yearling.

There is some fraternizing on the nesting grounds between the buffle-head and other ducks. It is not uncommon to see a female lesser scaup duck, usually a yearling, associating with a female buffle-head and brood while sometimes a yearling female buffle-head is seen with a brood of lesser scaup ducks.

Young buffle-heads sometimes follow a female, or male, of another species. Thus at 103 Mile Lake, August 8, 1936, a brood of 10 nearly full-grown young followed closely behind an adult, flightless male white-winged scoter, *Melanitta deglandi*, and continued to do so until the scoter dived and was not seen again. They also attract, or follow, broods of other species. For example, at Cummings Lake, August 10, 1940, two half-grown buffle-heads accompanied a brood of five lesser scaup ducks and at 105 mile Lake, August 5, 1940, a brood of nine was followed by five half-grown Barrow's golden-eye.

Sex Ratio

Determination of sex ratio is complicated by two factors (1) the similarity during autumn and early winter between males in first year plumage and females, (2) a partial segregation of adult males in early spring. In studying the sex ratio of autumn and winter flocks it has been observed that the number of adult males represented about 25% of the total. This was the case on November 6, 1940, in nearly all of 20 small flocks counted along 30 miles of sea coast between Departure Bay and Qualicum, Vancouver Island. If it is assumed that young males were present in about the same proportion as adult males a balanced sex ratio is indicated.

Counts made at the coast in March, when the identification of second year males presents little difficulty, show a preponderance of males:

		♂	♀
Esquimalt Lagoon	March 28, 1938	48	36
Departure Bay	March 25, 1938	60	40
Departure Bay	March 20, 1940	80	50
Hammond Bay	March 28, 1941	37	32

The following counts to determine the sex ratio were made on lakes where mating birds concentrate and the constitution of the flocks is constantly changing. It is possible that the excess of males indicated is a temporary seasonal condition corrected later with the arrival of additional females. On the nesting grounds during April adult males may outnumber females five or six to one; at this time few second year birds are present.

		♂	♀
103 Mile Lake	April 16, 1941	175	75
105 Mile Lake	April 16, 1941	35	15
Westwick Lake	April 18, 1941	10	4
Cummings Lake	April 18, 1941	40	16
Slough, 108 Mile Lake	April 22, 1941	16	4
Trapp Lake	April 24, 1941	16	4
Napier Lake	April 24, 1941	26	5

Moults and Plumages

In connection with this study 64 specimens in the author's collection, selected to represent both sexes from downy young to adult, have been available for reference. The specimens referred to below, by number, are in this collection. In addition, 122 other specimens comprising the collections of the Provincial Museum of British Columbia, the Royal Ontario Museum of Zoology, and the Canadian National Museum have been examined. The following is an attempt to interpret the plumage sequence on the basis of this material. The colour terms are those of Ridgway[1].

Downy young. The downy young buffle-head is white below and fuscous above with white markings on wings and white cheek patches; the fuscous colour of the natal down soon fades to a lighter, more brownish shade and by the time the first teleoptile shafts appear it has become still paler. In general appearance it closely resembles the similar stage of *Glaucionetta;* a constant differentiating character, perceptible only in freshly taken specimens, being the colour of the foot which in *Charitonetta* is dark plumbeous and in *Glaucionetta* deep olive.

♂ *Juvenals, July–August.* Chest, neck, upper tail coverts, and sides of head nearest to hair brown; indistinct white patch on cheeks and indication of white on throat; crown and interscapular region fuscous, intervening area on neck paler; wings black, except for larger secondaries and white markings on middle secondaries; larger secondaries olive-brown fading to drab and forming distinct *V* on back; tail fuscous, fading to pale brown; flanks and lower tail coverts hair brown to drab; lower chest and belly white. Fresh colours are evanescent and quickly modified by breaking down of the feather structure.

♂, *First autumn and winter, September–February.* During the first autumn the rectrices and contour feathers are gradually renewed. No. 4732, September 28, 1936, is in a state of complete body moult, the new rectrices about half-grown. The head becomes fuscous, the cheek patches are larger and more clearly defined than before; the chest is lighter in colour, pale smoke gray feathers with white edgings; the back is uniformly black, fuscous or deep

[1] *Ridgway, R. Color standards and color nomenclature. Published by the author. Washington, D.C. 1912.*

mouse gray with feathers on interscapular region tipped with lighter shade; flanks are solidly mouse gray; the tail, nearest to neutral gray, has a silvery sheen.

Some individuals retain this plumage with little modification until well into the winter. Thus No. 4518, January 12, 1935, is similar to specimens taken in October except for the presence of several white flank feathers. Others as early as November show modification brought about by moult. For example No. 5194, November 13, 1939, is conspicuously lighter than the January specimen referred to, a condition produced by the presence of white feathers on scapular regions and head, and white and light grey feathers edged with black on the flanks; this specimen also has conspicuous violet and bronze feathers on the head.

♂, *Spring and summer, second year.* As spring advances they become lighter in colour partly through the appearance of white feathers on flanks, scapulars, and head, partly through the breaking down and fading of the old plumage. Later, white middle wing coverts appear and these with the white middle secondaries form a conspicuous bar across the black wings (Fig. 5). The remiges become much abraded and fade to drab; in some they degenerate to a condition that impedes or prevents flight.

Three specimens in the author's collection, No. 5423, June 20, 1941, No. 5424, June 27, 1941, No. 5425, July 7, 1941, are in a full moult of the body plumage. On the ventral surface the vanes have broken down revealing the darker basal portions and shafts of the feathers so that the colour in the mass is pale smoke gray rather than white; in each the throat is white or buff, the chest drab gray or smoke gray, the rectrices, primaries, and secondaries light drab; the tips of the feathers on the interscapular region are faded, in one specimen forming a regular pattern. All these modifications have been produced by feather disintegration which also has altered the character of the violet-green feathers on the head and darkened the white areas of the same region. New feathers appearing on the lower chest and belly are white, those on the upper chest are light mouse gray, and those on the flanks are deep mouse gray. The residue of old feathers on the flanks is pale gray, white, and white edged with black. In the tail of one specimen, in which some of the abraded rectrices have broken off, is one new adult feather.

At the time these specimens were taken the few adult males present showed no indication of moult.

Three other specimens, No. 5009, August 4, 1938, No. 5281, August 8, 1940, and No. 5178, August 23, 1939, have faded on head, flanks, tail, and back to such an extent that the entire dorsal surface is mottled buff and brown of various shades. In one, the wings feathers have been shed and the new primaries are one inch or less out of their sheaths. In none is there a recognizable indication of eclipse plumage. After the second year moult the young male is probably indistinguishable from older males.

♂, *Summer plumage.* The five specimens described above are identified as being in their second year. The age of seven other specimens taken later in the season, no two of which are exactly alike, is not readily apparent; they are identified provisionally as adult males. These were taken on the following dates: No. 5280, August 4; No. 5283, August 12; No. 5282, August 14 (1940); No. 5177, August 22, 1939; No. 5317, 5318, October 2, 1940; No. 4430, October 4, 1935. The October specimens are evidently examples of retarded moult; each of two had a broken humerus recently healed and the third was emaciated and apparently diseased.

All were in a flightless condition. In No. 5280 and No. 5285 this was due to degeneration of the old flight feathers; in the remainder the flight feathers had been shed and the new feathers were not grown sufficiently to permit flight. In five specimens the rectrices are broken and faded drab to olive buff and in two of these is a single perfect, but not freshly acquired, feather. Another specimen has three feathers of this same type and in another all the old rectrices have been moulted and the new ones, half an inch in length, are concealed by the tail coverts.

In all specimens the ventral surface is pale mouse gray with areas of white where new feathers have appeared; the feathers on flanks are various shades of grey and fawn and in three specimens are several white feathers; the throat is white more or less obscured with a dusky shade; the heads are dusky with faded white cheek patches, in one specimen these and the white throat are confluent; the dorsal surface is mottled fawn, brown, and black, the brown tips of the black feathers on interscapular region forming in one case a definite horizontal pattern, in two others the fresh black secondaries are conspicuous against the brownish background; four have greenish reflections on the occiput, another has definite green and violet areas on cheek and chin.

♂, *Adult.* The adult male wears the familiar plumage, black and white body, white, violet, and green head for nine or possibly 10 months. There is some variation in the time during which individual birds moult but with the greater number it would appear to take place between early July and late August. As stated elsewhere most of them disappear at this time and they do so before any evidence of moult is visible. A specimen taken June 18, 1941, is in full plumage except for slight modification produced by wear. After the flight feathers have been resumed the moult of the body plumage continues for some time and a few adult males may reappear on the nesting grounds before they have attained full plumage. Thus at 103 Mile Lake, August 24, 1937, 22 males in this condition were counted. Early records for fully moulted males are: August 25, 1939; September 15, 1915.

♀, *Juvenals, July–August.* Similar, except for smaller size, to juvenal males. Much browner in general appearance than adult females. The original colours are soon modified. No. 5278, August 14, 1940, has faded to drab and various shades of light brown on the dorsal surface; the tail is cream; on the buff-coloured flanks several new feathers are mouse gray; only the flight feathers retain the original colour and texture.

♀, *First autumn, September–October.* As with the young males there is a gradual moult commencing in the late summer which renews all the plumage except the flight feathers. Thereafter young females more closely resemble adult females, the head and neck are browner, the chest band more fawn than gray, and usually the feathers on dorsal surface are tipped with fawn or gray. There is much individual variation in these indications of juvenility.

♀, *First winter and spring, November–June.* As winter progresses a degeneration in feather structure and loss of colour takes place which at first is most apparent in the tips of the flight feathers and later, on the dorsal surface where the lighter coloured feather edgings become more conspicuous. By May or June the colours have faded to such an extent that second year females may be identified in life with some degree of certainty. Later in the summer the difference in colour between yearling females and older females becomes still more apparent. No. 5008, second year female, August 4, 1938, has faded on the dorsal surface to various shades of brown; cream edgings of feathers on interscapular region form a definite pattern; amongst the old flank feathers, faded almost to white, new gray feathers are conspicuous. In August there is a complete moult, much of the faded body plumage and the faded rectrices being retained until the flight feathers are renewed. On completion of this moult the second year female, then approximately 15 months old, is indistinguishable from the adult female.

♀, *Adult.* The adult female moults at approximately the same time as the second year female. Thereafter the slate, dusky, and white plumage shows little modification until the following summer when some degree of fading takes place. In winter the feathers on the back present a solid coloured surface with bronze reflections in some specimens; the chest band is nearest to neutral gray.

Pattern on greater wing coverts. Young males may have the greater wing coverts above the five or six white middle secondaries unmarked or they may be variously patterned with white. In some this pattern is in the form of an isolated white spot on one, two, or more feathers, in others the terminal third or terminal half of each is white, in others most of one side of the shaft is white or the white is bordered by a dusky tip. In the latter this arrangement forms a narrow bar across the white wing patch (Figs. 7, 8, 9). The same range of variation occurs in the female as in the young male. The pattern appears to be an individual character that is not modified by age (Figs. 10, 11, 12).

Colours of Soft Parts

The colour of the iris in both sexes of all ages is dark brown. In the live adult male in winter and spring the tarsus is alizarine pink; after a bird has been killed and the blood drains from the tarsus it becomes decidedly paler. The following colour notes are from freshly taken specimens.

♂, ♀, *Downy young, approximately two days.* Bill dark neutral gray; nail pale gray; tarsus dark plumbeous.

♀, *Downy young, approximately 12 days.* Bill dark neutral gray; tarsus deep plumbeous.

♂, ♀, *Juvenals, approximately two months.* Upper mandible dark neutral gray; ramus dark Payne's gray; tarsus plumbeous.

♂, *First winter, December.* Sides and basal half of bill dark gull gray; top of bill nearest to olive-gray; interramus purplish gray; tarsus light quaker drab; webs dark quaker drab.

♀, *First winter, December.* Upper mandible neutral gray clouded with deep neutral gray; ramus purplish gray; tarsus light purplish gray.

♂, *Second year, June.* Upper mandible dark neutral gray; ramus dark gull gray; tarsus vinaceous drab inside, dark quaker drab outside.

♀, *Second year, August.* Bill dusky neutral gray; nail paler; tarsus vinaceous slate.

♂, *Eclipse.* Bill darker than in summer; upper mandible neutral gray; ramus dark gull gray; tarsus vinaceous pink.

♂, *Adult, November.* Upper mandible light Payne's gray, slate gray at base; tip of nail black; ramus pale Payne's gray; tarsus, webs, vinaceous fawn.

♀, *Adult, November.* Upper mandible slate colour; ramus light Payne's gray; tarsus pale Varley's gray; webs dark neutral gray.

Food and Feeding Habits

In its feeding habits the buffle-head is not gregarious to the same extent as are scaups and some other diving ducks. Although the population of a feeding ground may number several hundreds it is usual for them to be scattered over the area in small flocks. Many associate in pairs and trios and these seem more vagrant in their movements than are the larger units.

Food is secured almost entirely by diving, the time of submergence ranging between 15 and 35 sec. Sometimes the members of a small flock dive in quick succession so that for a short time all are submerged, or the dives may be so spaced that some birds always are on the surface.

On the sea they show preference for feeding inshore on a rising tide and are conspicuously active when a light surf is breaking close to the beach. Under such conditions some may ride for a short distance on the crest of a wave and as it tumbles into foam avoid the disturbance by a quick dive into calmer water. The sea immediately outside a breaking surf is a profitable feeding ground rich with multitudes of small animals that have been dislodged from their lurking places.

Following the salmon runs on the coast, small numbers of buffle-head frequent the salmon streams and feed on the uncovered salmon eggs. When disturbed they rise quickly and in rapid flight pass down stream to the sea. They fly not far above the surface and well below the tops of the alders that line the banks of most of these streams. They follow each turn of the stream

and seldom short cut over the tree tops as other ducks frequently do under similar circumstances. Buffle-heads also have been seen, in company with other waterfowl, feeding on the eggs of Pacific herring, *Clupea pallasii.*

Food Summaries

The following section summarizes the food eaten by 133 buffle-heads in various localities in the interior and on the coast of British Columbia; the figure following the month indicates the number of specimens examined.

Food of Downy Young

Peace River District: Tupper Creek, June, 2.

Cariboo region: Cummings Lake, June, 1; Lily Pad Lake, July, 1; Tatton Lake, June, 7, July, 2; 105 Mile Lake, June, 1, July, 5; Sheridan Lake, July, 1.

Amphipods. Amphipod fragments constituted 40 to 97% of the total contents of five specimens from 105 Mile Lake.

Insects. Seventeen of the 20 specimens examined had eaten insects, chiefly aquatic nymphs and larvae. In 13 specimens this was the exclusive item; in three others, insects represented 50, 70, and 98% of the total stomach contents. The following occurred the number of times indicated: Corixidae, 6; Chironomidae, larvae, including *Chaoborus* sp., 4, adults, 1; Coleoptera (terrestrial), 3; Coleoptera (aquatic), adults and larvae, including *Dytiscus* sp., *Haliplus leechi*, *Thermonectes* sp., 15; Odonata, dragonfly nymphs, 5, damsel fly nymphs, including *Enallagma* sp., 7; unidentified Diptera, 1; caddis, 2.

Seeds. Thirty seeds of *Polygonum hydropiper* composed 50% of the contents of one stomach; two seeds of *Potamogeton pusillus* constituted a minor item in another.

Miscellaneous vegetation. In three stomachs, unidentified plant material formed 10, 50, and 60% of the contents.

TABLE I

Food of 20 downy young buffle-heads, total percentage volume

Locality	Number of specimens	Amphipods	Odonata	Coleoptera	Corixids	Miscellaneous insects	Miscellaneous vegetation
Tupper Creek	2		31.50	50.00	15.00	3.00	0.50
Lily Pad Lake	1			50.00		50.00	
Tatton Lake	9		40.00	45.67	2.33	12.00	
105 Mile Lake	6	59.50	16.33	1.00	3.00	0.17	20.00
Sheridan Lake	1			40.00		10.00	50.00
Cummings Lake	1			35.00	50.00	15.00	

Food of Adults

Interior Region

Cariboo

Rush Lake, Springhouse, June, 3; 103 Mile Lake, June, 1, October, 2; 105 Mile Lake, August, 6, October, 3; Watson Lake, August, 1; Mirage Lake, August, 1; pond, Lac La Hache, August, 1; Disputed Lake, September, 4; Longbow Lake, September, 8; Lone Butte Lake, September, 2.

Amphipods. Amphipods composed 60 and 70% of the food contents of each of two stomachs from 105 Mile Lake and 60% in another specimen from Disputed Lake.

Aquatic insects. Aquatic insects were present in all but two of the 32 stomachs examined, the most important in volumetric percentage being Odonata. Damsel fly and dragonfly nymphs each occurred in 16, the latter representing 59% of the total volume of food in 12. One bird had eaten over 70 specimens of *Enallagma* sp.; corixid debris was detected in 23 stomachs. In one stomach *Culex* larvae constituted 45% and in another, 5% of the food eaten. Other insects were identified the number of times indicated, viz., May fly nymphs, 3; caddis larvae, 6; dipterous larvae, 2; chironomid larvae, 3.

Coleoptera. Fragments of terrestrial beetles were present in five stomachs and in each represented a minor percentage of the food eaten.

Molluscs. Gastropod fragments were detected in five stomachs; in two composing 50% of the total food content.

Miscellaneous animals. Bryozoan statoblasts were present in six stomachs, in one constituting 89% of the food eaten. One contained a small amount of sponge (Porifera) another, traces of a phyllopod and two others, several leech egg cases.

Algae. A small amount of the filamentous algae, *Spirogyra* and *Zygnema*, was contained in one specimen.

Seeds. Seeds formed, in most cases, a minor item of the total food in a stomach. The most important were *Potamogeton* species and *Myriophyllum;* one contained 50 seeds of *Polygonum* sp. The plants represented were: *Sparganium* sp., *Potamogeton pusillus*, *P. heterophyllus*, *P. pectinatus*, *Zannichellia palustris*, *Najas flexilis*, *Carex* sp., *Scirpus* sp., *Rumex* sp., *Polygonum amphibium*, *Polygonum* sp., *Myriophyllum spicatum*.

Miscellaneous vegetation. Small quantities of unidentified plant material were present in two stomachs.

Okanagan

Swan Lake, September, 3, October, 5, November, 15, December, 4.

Fishes. Fragments of a small sculpin, *Coltus asper*, composed 25% of the food in one December specimen.

Crustaceans. Amphipods constituted from 25 to 60% of the food in four and smaller percentages in nine other stomachs. Both *Gammarus limnaeus* and *Hyalella azteca* were identified; one contained over 165 of the latter. One isopod, sow bug, had been eaten.

Aquatic insects. Insect material was present in 22 of the 26 stomachs examined and in 17 composed 25% or more of the total food content. The following were represented the number of times indicated: May fly nymphs, 6; dragonfly nymphs, 9; damsel fly nymphs, 9; corixids, 21; microcaddis pupae, 6; Coleoptera, adults, *Haliplus leechi*, *Dytiscus* sp., 2; Coleoptera, larvae, including *Dytiscus* sp., 4; chironomid larvae, 10.

Molluscs. A large number of gastropods, *Helisoma trivolvis*, were present in one, several *Lymnaea* sp. in another, and *Planorbis* sp. in three other stomachs.

Miscellaneous animals. Bryozoan statoblasts in five stomachs represented small percentages; in another five leeches averaging 50 mm. composed 50%, and in a third four leeches averaging 55 mm. composed 70% of the total food.

Algae. A small quantity of *Chara* oospores was in one stomach.

Seeds. Seeds of aquatic plants, usually in small amounts, were present in 19 stomachs. One bird had eaten over 200 *Scirpus* seeds, another 61 seeds of *Potamogeton pectinatus*. Seeds of *Zannichellia palustris*, *Ceratophyllum demersum*, and *Potamogeton heterophyllus* also were identified.

Miscellaneous vegetation. Leaves of *Ceratophyllum demersum* were detected in one specimen; small amounts of unidentified plant material occurred in three others.

Okanagan Lake, January, 16, February, 2, March, 1, September, 1, November, 1, December, 2.

Fishes. Bones and other fragments of sculpins, probably *Cottus asper*, occurred in five stomachs and in two of these composed 82% of the total food content.

Crustaceans. Traces of crustacean material were detected in two stomachs.

Aquatic insects. Caddis larvae represented small percentages in 13 stomachs. Ephemeridae (debris), in one case noted as representing about 40 nymphs and in another composing 80% of the total food, were present in seven stomachs. One bird had eaten 275, another 280, chironomid larvae. Corixids were present in three, Dytiscidae, larvae, were minor items in two, and insect debris, not further identified, occurred in nine stomachs.

Molluscs. Molluscs either univalves or bivalves, or both, were present in 14 stomachs with a total percentage volume of 38.52. *Lymnaea vahli* and *Planorbis* sp. were the only identifications.

Miscellaneous animals. Items included under this heading include animal matter, not further identified, that was present in five specimens.

Algae. One stomach contained approximately 6500 *Chara* oospores, another a small amount of *Chara* branches, and a third a small quantity of unidentified algae.

Seeds. Seeds of *Najas flexilis* occurred in nine stomachs; one contained 235 representing 23% of the total food. *Scirpus* seeds were present in 11 and *Potamogeton* seeds in nine. Species of less importance were *Polygonum* sp., *Spartina* sp., *Rubus* sp.

Miscellaneous vegetation. Plant fibre and ground up tubers of *Potamogeton* species constituted 75 and 90% of the contents of two and were identified in two other stomachs. *Najas* fibres occurred in two and small amounts of unidentified plant material in 15 other stomachs.

Vernon Commonage ponds, June, 1, September, 1, October, 5; Pond, Trinity Valley, November, 1.

Crustaceans. One occurrence of an amphipod and *Daphnia* constituted 1% of the food in each of two stomachs.

Aquatic insects. Aquatic insects composed 90.57% of the total food in the stomachs of seven specimens from the Vernon Commonage ponds. One had eaten 280 chironomid larvae, another, a large number of dragonfly nymphs and a third, damsel fly nymphs. *Notonecta* sp. and corixids constituted 90% of the food in the September specimen; the latter composed 50% of the contents in another and were present in four other stomachs.

Miscellaneous animals. Water mites, Hydrachnidae, occurred twice as minor items. The specimens from Trinity Valley had eaten over 1500 bryozoan statoblasts that constituted 75% of the total stomach contents.

Seeds. Seeds of *Potamogeton* sp., *Scirpus* sp., and *Bromus* sp. were minor items in three, 20 seeds of *Potamogeton pectinatus* formed 10% of the food in another stomach.

Miscellaneous vegetation. Plant debris, including *Potamogeton* plant fibre and tubers constituted 15 and 19% of the contents of two and were minor items in three other stomachs.

Coast Region

Fresh Water Habitat

Lower Fraser River, February, 1, November, 1, December, 1; Henderson Lake, November, 1; Quennell Lake, January, 1; Cowichan River, January, 3.

Salmon eggs. The specimen from Henderson Lake contained 25 eggs of sockeye salmon, *Oncorhynchus nerka* as the exclusive item.

Fishes. Rostra and other bones of stickleback, *Gasterosteus aculeatus*, were present in two stomachs from the mainland and in one from Vancouver Island. In one instance this item consisted of approximately 12 and in another of six fishes. Bones of an unidentified fish composed 60% of the contents of a specimen from Cowichan River.

Aquatic insects. Twelve fly larvae (Brachycera) constituted the chief food item in the specimen from Quennell Lake which also contained fragments of corixid adults, chironomid and caddis larvae. One from the lower Fraser River contained debris representing an adult aquatic beetle, corixids, and caddis; in two others, corixids composed 70 and 75% of the contents. At least 200 caddis larvae (Phrygancidae) was the only food in one specimen from Cowichan River.

Seeds. Seeds of *Scirpus* sp. were present in five stomachs, in one case 30, in another 50, had been eaten. Other species represented were *Echinochloa crusgalli*, *Ceratophyllum demersum*, *Potamogeton pectinatus*, *P. foliosus*.

Salt Water Habitat

Kemp Creek, December, 1; Boundary Bay, December, 2; Cowichan Bay, January, 1; Ladysmith Harbour, January, 1; Departure Bay region, December, 5, January, 5.

Fishes. The abraded bones of small marine fishes constituted 60% of the contents of one; a pipe fish, *Syngnathus griseo-lineatus*, 155 mm. in length, was the chief item in a second and bones of a small sculpin occurred in a third specimen.

Crustaceans. Two stomachs contained isopods as the chief item, in one case *Pentidotea* sp., in the other *Exosphaeroma oregonensis*. Amphipod debris was present in five specimens, in one composing 95% of the contents. Fragments of shore crabs, *Hemigrapsus nudus*, were the sole item in two, 98% in another, and represented small percentages in four other stomachs; a small hermit crab, *Pagurus* sp. and several mysids also had been eaten.

Molluscs. Molluscs were the sole item in two, 70 to 90% in six, and small percentages in four other stomachs. One bird had eaten eight tectibranchs that averaged 15 mm. in length and another contained a few barnacle scutes. Species were identified as *Littorina scutulata* Gld., *Colliostoma* sp., *Columbella gausapata* Gld., *C. permodesta* Dall, *Bittium* sp., *Odostomia* sp., *Turbonella* sp., *Thais lamellosa* Gmelin, *Margarites pupilla* Gld., *Alectrion mendicus* Gld., *Lacuna divaricata* (Fab.), *Saxidomus giganteus* (Desh.), *Cardium corbis* Mart., *Paphia staminea* Con. None of the bivalves measured more than 5 mm. in length.

Miscellaneous animals. Jaws of a marine worm *Nereis* sp. was a minor item in a well-filled stomach.

Algae. Marine algae represented five to 10% of the contents of four stomachs.

Seeds. One seed of *Polygonum* sp. in the specimen from Kemp Creek represented the only plant material in this series of specimens.

Summary

Aquatic insects formed the chief food of 13 downy young from five small Cariboo lakes; on another lake amphipods were of first importance. Plant

material was represented in three stomachs, in one forming 50% of the total contents.

Aquatic insects provided the principal food in the summer and autumn diet of 32 adults and full-grown young taken in the Cariboo region and of eight from comparable waters in the Okanagan region. This item composed over 50% of the food eaten by 27 on Swan Lake during the period September 28 to December 5 but was a less important constituent in the food of 30, chiefly winter-taken specimens, from Okanagan Lake. At Swan Lake seeds of aquatic plants were second and amphipods, third in importance. On Okanagan Lake molluscs become more prominent with a total percentage volume of 38.52 and plant food is eaten to a greater extent than elsewhere. Seeds of aquatic plants are represented in small quantities in the majority of specimens from most localities. Chara oospores occurred only once as a major item.

From observation on the coast region it was determined that in some waters salmon eggs are sought for; the one stomach available from a spawning area contained this food exclusively. The food of chief importance on coast lakes and streams is aquatic insects; it is probable that small fishes are taken to a greater extent than the data indicate. Vegetable food is eaten in relatively small amounts. On salt water crustaceans and molluscs are about equal in times of occurrence and total percentage volume. Small fishes are of secondary importance. Herring eggs are eaten in the spring chiefly during the month of March.

TABLE II

FOOD OF BUFFLE-HEAD, TOTAL PERCENTAGE VOLUME

Locality	Number of specimens	Salmon eggs	Fishes	Crustaceans	Insects	Molluscs	Miscellaneous animals	Algae	Seeds	Miscellaneous vegetation
Rush Lake	3				20.00				80.00	
103 Mile Lake	3				46.66	16.67			30.67	6.00
105 Mile Lake	9			14.40	52.60	9.00	11.00	1.12	10.23	1.65
Watson Lake	1				96.00				4.00	
Mirage Lake	1				100.00					
Pond, Lac La Hache	1				100.00					
Disputed Lake	4			15.00	72.00		5.00		8.00	
Longbow Lake	8				94.00		1.00		5.00	
Lone Butte Lake	2				95.50		2.50		2.00	
Swan Lake	27		0.93	9.41	55.44	4.07	5.15	0.11	21.48	3.41
Okanagan Lake	23		7.83	0.09	20.30	38.52	3.91	3.65	4.27	21.43
Commonage Ponds	7			0.29	90.57		0.29		2.71	6.14
Lake, Trinity Valley	1				25.00		75.00			
Lower Fraser River	3		8.00		56.66				35.34	
Henderson Lake	1	100.00								
Quennell Lake	1		10.00		89.00				1.00	
Cowichan River	3		20.00		66.67				13.33	
Kemp Creek	1			99.00					1.00	
Boundary Bay	2		2.50	46.00		48.00		3.50		
Cowichan Bay	1			85.00		15.00				
Ladysmith Harbour	1					100.00				
Departure Bay	10		7.50	50.90		39.50	0.10	2.00		

Economic Status

Like may other species of waterfowl the buffle-head is known to feed upon the eggs of Pacific salmon and Pacific herring. The facts are not in dispute but it seems doubtful if the amount of ova consumed could affect the results of a spawning of either salmon or herring. The number of birds involved is small; the total of eggs available during and after a spawning is vast and of these an uncalculated but probably large percentage becomes a waste product upon which many kinds of birds and fishes feed.

No evidence of buffle-heads eating the fry of salmon or trout has been obtained and their consumption of other small fishes represents a minor item of diet. Stickleback, sculpin, pipe fish, and an unidentified marine fish had been eaten by five of the 15 specimens taken on coast waters and nine of the 118 specimens taken in the interior contained sculpins. Bent (3) cites three authors who refer to small fishes as food items in eastern North America; in one case trout fry is mentioned. Cottam (1) found fish remains representing a total percentage of 3.78 in the total of food eaten by 282 adults from many localities, the most valuable species being one small eel and a sunfish.

The value of the "butterball" as a game bird is less than that of many other ducks, nevertheless it is of some importance. Considered from the standpoint of food it is highly rated as a table bird by the experienced wild fowler in the interior. On the coast where a diet of marine organisms imparts an unpleasant flavour to the flesh it is not so regarded.

Considered from the point of view of "sport" its pursuit attracts chiefly the less experienced hunter. Usually it is the tamest of the ducks and will alight amongst a flock of decoys which may have failed to attract the more desirable species. It has the habit of flying along a lake shore within easy shotgun range and a flock may return to pass a given point several times during a few hours until many of its members have been killed. Nevertheless hunting pressure is not an important reducing factor under normal conditions but in years when the general duck population is below average the pressure is increased to a point that might seriously reduce the population. This was the case in the middle 1930's when drought conditions on the nesting grounds greatly reduced the numbers of those species that the hunter considers most desirable.

The buffle-head possesses in a high degree those qualities of beauty and behaviour that render it an object of affection to many. This is a point that must be considered in an evaluation of the economic status of the species.

From the foregoing it can be concluded that (1) any possible reduction in commercial fish populations is negligible and (2) that the species is otherwise of aesthetic and economic importance.

Acknowledgments

Grateful acknowledgment is made to the United States Fish and Wildlife Service for permitting the use of their original records of stomach analyses of specimens collected by the author during the years 1911 to 1918; to Dr. R. E. Foerster, Director, Pacific Biological Station, and to the Fisheries Research Board of Canada for the use of laboratory facilities; to the National Museum of Canada, the Royal Ontario Museum of Zoology, and the Provincial Museum of British Columbia for permission to examine specimens; to Mr. W. B. Johnstone and Mr. Allan Lyon for permission to use unpublished migration records; to the Entomological Branch, Department of Agriculture, Ottawa, Canada, Dr. G. Clifford Carl, Dr. Josephine F. L. Hart, and Mr. D. Quayle for the identification of certain food items; to Mr. K. Racey, Mr. A. Peake, and Dr. M. Y. Williams for contributing buffle-head stomachs and to Dr. Ian McTaggart Cowan for photographing the series of buffle-head wings, Plate II.

References

1. COTTAM, A. C. Geo. Wash. Univ., Summaries Doctoral Theses, pp. 66-70. 1937.
2. COWAN, I. McT. British Columbia Provincial Museum, Occasional Papers, No. 1. 1939.
3. BENT, A. C. U.S. Natl. Museum, Bull. 130. 1925.
4. KELSO, J. E. H. Ibis, 2 : 689-723. 1926.
5. MUNRO, J. A. Condor, 39(4) : 163-173. 1937.
6. Munro, J. A. Trans. Roy. Can. Inst. 22(2) : 259-318. 1939.
7. TAVERNER, P. A. Condor, 21(2) : 80-86. 1919.
8. SWARTH, H. S. Univ. Calif. Pub. Zool. 10(1) : 1-124. 1912.
9. SWARTH, H. S. Univ. Calif. Pub. Zool. 24(2) : 125-314. 1922.
10. SWARTH, H. S. Univ. Calif. Pub. Zool. 24(3) : 315-394. 1924.
11. SWARTH, H. S. Proc. Calif. Acad. Sci. (Ser. 4) 23(2) : 35-58. 1936.

PROTEUS HYDROPHILUS INFECTIONS OF PIKE, TROUT, AND FROGS[1]

By G. B. Reed[2] and G. C. Toner[3]

Abstract

Proteus hydrophilus has been isolated from and shown to be the probable cause of "ulcer disease" of hatchery reared brook trout.

Cultures of *Proteus hydrophilus* isolated from "red sore" diseased pike, "ulcer" diseased trout, "red leg" diseased frogs and from lake water are shown to be indistinguishable by cultural and biochemical reactions.

Proteus hydrophilus infections are shown to be transmissible by water.

In a recent paper by Reed and Toner (3) it was shown that a prevalent disease of adult pike in eastern Ontario waters, known locally as "red sore" is caused by an infection with *Proteus hydrophilus*. It was pointed out that this organism has long been known as the cause of the widespread "red leg" disease of frogs and that the work of Fish (2) associated it with "ulcer disease" of young trout in a New York State hatchery.

1. *Proteus hydrophilus* in Trout

Through the kindness of Mr. W. H. R. Werner of the Ontario Department of Fisheries, the authors recently had an opportunity to examine young brook trout in a small temporary hatchery in eastern Ontario where an epizootic of ulcer disease was in progress, resulting in several hundred deaths per day. The gross lesions in these young trout, 2 to 3 in. in length, apparently fit the descriptions given by Calkin (1) and Fish (2) for ulcer disease as seen in New York State hatcheries. At death the fish had one or more lesions from a few millimetres to a centimetre in diameter, varying from a gray white induration of the skin to deep, necrotic, reddened ulcers extending through the skin into the musculature. These appeared on any part of the body of the fish. The visceral organs, on gross examination, appeared normal except that the kidneys were generally engorged and occasionally necrotic.

The gross appearance in the young trout is very similar to the red sore condition of adult pike except that in the latter species the sores tend to be more superficial with a wider area of infiltration and reddening.

Sixteen trout with conspicuous ulcers, but still living when taken from the tanks, were examined in some detail for parasitic organisms. The culture procedure was that previously followed for pike (3). *P. hydrophilus* was recovered from the necrotic lesions of all 16 fish, from the kidney of six, and from the body fluid of one. All the cultures gave the characteristic reactions of *P. hydrophilus*, Section 5.

[1] *Manuscript received January 19, 1942.*
Contribution from Department of Bacteriology, Queen's University, Kingston, Ont.

[2] *Professor of Bacteriology.*
[3] *Research Assistant.*

The authors have not had an opportunity to test the pathogenicity of the cultures on young trout; however they were tested in goldfish which had previously been found to be easily infected with strains from pike (3). Of the 40 strains, isolated from 16 diseased trout, 10 were selected at random for pathogenicity tests. Twenty-four–hour cultures in broth were injected intraperitoneally into goldfish in 0.1 and 0.01 cc. amounts. The results, summarized in Table I, indicate that all strains tested were pathogenic for goldfish.

TABLE I

PATHOGENICITY IN GOLDFISH OF 10 STRAINS OF *P. hydrophilus* ISOLATED FROM TROUT

Source	Dose of 24-hr. culture	
	0.1 cc.	0.01 cc.
7 strains isolated from skin lesions	Dead in 18 hr. or less	Dead in 48 hr. or less
1 strain isolated from kidney	Dead in 16 hr.	Dead in 30 hr.
2 strains isolated from kidney	Dead in 48 hr.	Not killed.

The fact that *P. hydrophilus* was isolated from the ulcers of all the trout examined and that representative cultures are pathogenic for goldfish makes it highly probable that this is the causal organism of the trout disease. This is in agreement with the conclusion reached by Fish (2).

The origin of the infection in the hatchery was not established. In another section it has been suggested that this organism may be transported by water from a possible reservoir in diseased frogs. But an examination of the water entering this hatchery through a pipe line from a small spring-fed reservoir failed to reveal *P. hydrophilus*.

2. *Proteus hydrophilus* in Lake Water

The evidence appears conclusive that *P. hydrophilus* is the causal agent of "red sore" of pike, "ulcer disease" of trout, and "red leg" of frogs. There is less conclusive evidence that it produces ulcer-like lesions in suckers, bass, perch, and pickerel. It would be of interest to know how it is distributed. To this end the water of some 14 lakes and streams in eastern Ontario was examined for the presence of *P. hydrophilus*. Pike in all of these lakes were known to be infected to a greater or less degree by red sore and frogs in swamps bordering part of the lakes were known to be infected with red leg; the prevalence of red leg in the region suggests that frogs in all the lakes examined were probably infected.

The procedure followed in examining the water consisted in making serial dilutions of the water in sucrose broth. Any that failed to show sucrose fermentation with the largest inoculum, 1 cc. of water, were regarded as free from *P. hydrophilus*. Where fermentation occurred the tube inoculated with the smallest volume of water showing the fermentation was plated on sucrose–brom-thymol-blue agar from which sucrose fermenting colonies were isolated

for detailed identification (3). Cultures isolated in this manner were subjected to the usual biochemical reactions for the identification of *P. hydrophilus* and tested for pathogenicity in goldfish.

Of the 14 lakes and streams examined, four yielded cultures of *P. hydrophilus*. These cultures were indistinguishable from cultures isolated from pike, trout, and frogs (Section 5). All four cultures were pathogenic in goldfish, the infections being fatal. It is possible that a more detailed examination of the water might have indicated a wider distribution. It is, however, significant that the organism was isolated from 4 to 14 samples tested.

These results suggest that the mode of distribution of *P. hydrophilus* to fish is via water, possibly from a reservoir in diseased frogs.

3. Experimental Water-borne Infection

A group of 50 goldfish were exposed to water-borne *P. hydrophilus* infection in the following manner. The goldfish had been for some two months in a cement tank 6 × 3 ft. containing water to a depth of 8 in. Approximately 1 gal. of fresh water per hour was added from a dripping tap. An overflow pipe kept the level constant. There had been no casualties for two months.

Two goldfish infected with *P. hydrophilus* and in a moribund condition were put in the tank. They died within the next few hours and the bodies were not removed until 48 hr. later.

During the next 16 days, six fish died, one on the seventh, ninth, tenth, twelfth, fifteenth, and sixteenth days, after the exposure began. In each instance *P. hydrophilus* was recovered from the kidneys of the dead fish. On the sixteenth day after the beginning of the exposure cultures were made from the slime of the remaining 44 fish. From 21 of the 44, *P. hydrophilus* was recovered and these cultures, injected intraperitoneally into other goldfish, produced fatal infections.

It is evident therefore that contact with two dead fish infected with *P. hydrophilus* in a relatively large tank supplied with flowing water resulted in fatal infections in some 12% of the fish. It is also apparent that the slime of almost 50% of the fish became infected without any noticeable deleterious effects.

4. Optimum Temperature for *Proteus hydrophilus*

If *P. hydrophilus* is distributed by water, as the two previous sections suggest, it is important to know the temperature–growth range of the organism. A series of tubes of sucrose broth were inoculated with six strains of *P. hydrophilus*, two recovered from pike, two from trout, and two recovered from lake water. The results of incubation of the cultures at temperatures from 6° to 40° C. are summarized in Table II. The amount of growth was estimated by comparing the opacity of the broth cultures. It is apparent from the table that growth occurs over a very wide temperature range: from slightly below 6° C. to slightly above 37° C. The optimum growth range, judged by the

TABLE II

TEMPERATURE RANGE FOR GROWTH OF *P. hydrophilus*

P. hydrophilus	6° C.			10° C.			15° C.	20° C.	37° C.		40° C.
	Incubation period, hr.										
	18	40	72	18	40	72	18	18	18	40	72
From Pike 1	−	−	−	+	++	+++	+++	+++	++	+++	−
2	−	+	++	+	++	+++	+++	+++	++	+++	−
From Trout 1	−	−	+	+	++	+++	+++	+++	++	+++	−
2	−	+	+	+	++	+++	+++	+++	++	+++	−
From Water 1	−	+	+	+	++	+++	+++	+++	++	+++	−
2	−	−	+	+	++	+++	+++	+++	++	+++	−

NOTE: (−) = *no growth.*
(+) = *very slight growth.*
(++) = *moderate growth.*
(+++) = *maximum growth.*

amount of growth at the temperatures tested, appears to be between 10° and 15° C. to approximately 30° C.

It is therefore apparent that, if other conditions are favourable, this organism may be expected to grow in lakes from shortly after the ice goes out until shortly before it forms.

5. Comparison of *Proteus hydrophilus* Isolated from Pike, Trout, Frogs, and Lake Water

The possibility of cross infections from pike, trout, or frogs and the possibility of transmission by water depends upon the identity of the infecting organisms in the different hosts. In order to determine the degree of similarity of *P. hydrophilus* from three host species and from water, a comparison has been made of some 17 cultures isolated from red sore of diseased pike taken in different lakes in eastern Ontario; 23 cultures isolated from ulcer disease of young trout in one eastern Ontario hatchery; three cultures isolated from young trout in a Nova Scotia hatchery and kindly sent by Dr. R. H. M'Gonigle of the Atlantic Biological Station; two cultures isolated from red leg of diseased frogs collected in Quebec; four cultures isolated from lake water (Section 3).

Biochemical reactions of the 49 cultures are summarized in Table III. It is apparent that all 49 cultures ferment sucrose, maltose, mannitol, glucose, fructose, trehalose, galactose, and mannose. All 49 fail to ferment lactose, inositol, raffinose, and dulcitol. The fermentation of starch, dextrin, sorbitol, and salicin is variable; about an equal proportion of cultures from the four sources ferment and do not ferment these compounds. All 49 cultures form hydrogen sulphide, form indole, and liquefy gelatine.

TABLE III

BIOCHEMICAL REACTIONS OF 49 CULTURES OF *Proteus hydrophilus* ISOLATED FROM PIKE, TROUT, FROGS, AND WATER. THE FIGURES REPRESENT THE NUMBER OF STRAINS THAT GIVE POSITIVE AND NEGATIVE REACTIONS

	Reaction	17 strains from pike	26 strains from trout	2 strains from frogs	4 strains from water
Lactose	+	0	0	0	0
	−	17	26	2	4
Sucrose	+	17	26	2	4
	−	0	0	0	0
Maltose	+	17	26	2	4
	−	0	0	0	0
Mannitol	+	17	26	2	4
	−	0	0	0	0
Glucose	+	17	26	2	4
	−	0	0	0	0
Levulose	+	17	26	2	4
	−	0	0	0	0
Starch	+	12	20	2	4
	−	5	6	0	0
Dextrin	+	11	18	2	1
	−	6	8	0	3
Inositol	+	0	0	0	0
	−	17	26	2	4
Raffinose	+	0	0	0	0
	−	17	26	2	0
Sorbitol	+	8	17	0	0
	−	9	9	2	4
Trehalose	+	17	26	2	4
	−	0	0	0	0
Galactose	+	17	26	2	4
	−	0	0	0	0
Mannose	+	17	26	2	4
	−	0	0	0	0
Dulcitol	+	0	0	0	0
	−	17	26	2	4
Salicin	+	9	15	2	2
	−	8	11	0	2
H_2S formation	+	17	26	2	4
	−	0	0	0	0
Indole formation	+	17	26	2	4
	−	0	0	0	0
Gelatine liquefaction	+	17	26	2	4
	−	0	0	0	0

The colonial form on nutrient agar shows some variation. The most usual form is an almost entire low convex colony with a very slightly granular surface. On moist agar, most colonies show a tendency to spread in the characteristic amoeboid fashion of the genus. Some strains show the amoeboid tendency to a greater degree than others but no differences of this type could be related to the source of the culture. Very old cultures show a marked tendency to vary from *S* to *R* forms but this again is unrelated to the source. In fluid media all strains form a more or less definite pellicle.

In a previous attempt to relate *P. hydrophilus* isolated from pike and frogs by their antigenic structure (3) it was shown that strains from pike are antigenically so heterogeneous that on this basis no relationship could be shown among cultures from similar hosts. Wenner and Rettger (5) and Taylor (4) found a similar heterogenicity to exist in other species of the genus *Proteus*. Accordingly, no further antigenic examinations have been made.

Acknowledgment

The writers are indebted to the National Committee on Fish Culture for financial assistance.

References

1. CALKINS, G. N. Fourth Ann. Rept. Comm. Fisheries, Games and Forests, New York State. 1899.
2. FISH, F. F. Trans. Am. Fisheries Soc. 64 : 252-258. 1934.
3. REED, G. B. and TONER, G. C. Can. J. Research, D, 19(5) : 139-143. 1941.
4. TAYLOR, J. F. J. Path. Bact. 31(4) : 897-915. 1928.
5. WENNER, J. J. and RETTGER, L. F. J. Bact. 4 : 331-353. 1919.

THE VITAMINS A AND D POTENCY OF THE OILS OBTAINED FROM THE LIVER, INTESTINES, BODY, AND OFFAL OF SHAD, *ALOSA SAPIDISSIMA* WILSON, AND MACKEREL, *SCOMBER SCOMBRUS* L.[1]

By L. I. Pugsley[2], J. T. Kelly[3], W. A. Crandall[4], and C. A. Morrell[5]

Abstract

Data are presented on the percentage of liver and intestines in the fish, percentage of oil in body, liver, intestines, and offal and the vitamins A and D potency, iodine value, and percentage of unsaponifiable matter in these oils of shad and mackerel.

In view of the increased demand for natural resources of vitamins A and D and since no data are available in the literature on the by-products of shad and mackerel, it appeared of interest to make a preliminary survey of the oils obtained from these fish caught from fishing districts in the Maritime Provinces of Canada.

Materials and Methods

Shad caught in the vicinity of Saint John, N.B., and mackerel in the vicinity of Halifax, N.S., were shipped to the Laboratory on ice. There the livers and intestines were removed and weighed and oils were obtained separately from the body, liver, intestines, and offal (heads, livers, and intestines). The vitamin A was determined by the antimony trichloride test using an Evelyn photoelectric colorimeter (3) and vitamin D figures were obtained by biological assay with rats using the line test. Three dosage levels of the samples and standard were employed using 10 rats for each level; the results were calculated according to Bliss and Marks (1, 2).

TABLE I

The weight and percentage of liver and intestines of shad and mackerel

Sample	Date caught	Number of fish	Weight, gm.		Liver in fish, %		Intestines in fish, %	
			Mean	*S.E.*	Mean	*S.E.*	Mean	*S.E.*
Mackerel (1)	2/7/40	42	651	± 34	2.1	± 0.11	3.7	± 0.16
Mackerel (2)	29/11/40	15	781	± 41	1.6	± 0.05	3.6	± 0.09
Shad (1)	20/5/40	14	1752	±143	1.3	± 0.16	3.1	± 0.22
Shad (2)	20/8/40	25	1158	± 26	1.4	± 0.08	4.2	± 0.14

[1] *Manuscript received March 17, 1942.*

Contribution from the Laboratory of Hygiene, Department of Pensions and National Health, Ottawa, Canada.

[2] *Pharmacologist.*

[3] *Senior Laboratory Assistant.*

[4] *Assistant Pharmacologist.*

[5] *Senior Pharmacologist.*

Results

The mean weight, percentage of liver and percentage of intestines in the fish, and the mean percentage of oil in these tissues are presented in Tables I and II, respectively. The vitamin A potency of individual samples and the

TABLE II

THE PERCENTAGE OF OIL IN THE BODY, LIVER, INTESTINES, AND OFFAL OF SHAD AND MACKEREL

Sample	Number of fish	Oil in body, %		Oil in liver, %		Oil in intestines, %		Oil in offal, %
		Mean	*S.E.*	Mean	*S.E.*	Mean	*S.E.*	Mean
Mackerel (1)	16	9.9	±0.9	9.2	±1.2	4.1	±0.4	—
Mackerel (2)	15	19.1	±2.1	16.1	±1.5	13.1	±1.2	16.6*
Shad* (1)	13	20.8	—	15.6	—	14.4	—	—
Shad (2)	12	18.9	±1.5	18.8	±2.8	30.9	±2.8	—

* *Duplicate determinations on samples from pooled lots of fish.*

TABLE III

THE VITAMINS A AND D POTENCY OF THE OIL OBTAINED FROM BODY, LIVER, INTESTINES, AND OFFAL OF SHAD AND MACKEREL

Sample	Individual samples			Pooled samples				
	Number of fish	Vitamin A, *L* value per gram*		Number of fish	Vitamin A, *L* value per gram*	Vitamin *D*, International Units	Iodine value	Unsap. matter, %
		Mean	*S.E.*					
Mackerel (1)								
Liver oil	16	87,100	±45,500	26	74,700	1035	151	.5
Intestinal oil	16	45,100	±21,000	26	57,900	76	160	9.0
Body oil	16	0	—	26	0	20	147	0.3
Mackerel (2)								
Liver oil	15	49,900	± 5900	—	—	—	—	—
Intestinal oil	15	5000	± 770	—	—	—	—	—
Body oil	15	0	—	—	—	—	—	—
Offal oil	—	—	—	30	950	51	140	1.4
Shad (1)								
Liver oil	—	—	—	11	6400	51	153	7.6
Intestinal oil	—	—	—	11	660	20	114	2.9
Body oil	—	—	—	11	0	32	122	0.4
Offal oil	—	—	—	7	160	41	124	5.3
Shad (2)								
Liver oil	12	3600	± 900	13	1500	100	153	3.9
Intestinal oil	12	500	± 170	13	550	21	138	1.0
Body oil	12	0	—	13	0	27	138	0.2

* *For the purposes of this report L value per gram is an approximation of the potency in International Units.*

vitamin A and D potency, iodine value, and percentage of unsaponifiable matter in samples of oil obtained from pooled lots of fish are presented in Table III.

Considerable variation in the vitamin potency was found for the individual samples, but the values obtained for mackerel liver oil were within the limits of those usually reported for halibut liver oil, and the shad liver oil approximated the potency of authentic cod liver oil. The body oil of both fish was devoid of vitamin A as shown by the antimony trichloride test. The intestinal oils of mackerel are relatively high in vitamin A but low in vitamin D.

Although the distribution obtained for individual values was not normal, the standard error of the mean is given to indicate the variation.

References

1. BLISS, C. I. and MARKS, H. P. Quart. J. Pharm. Pharmacol. 12(1) : 82-110. 1939.
2. BLISS, C. I. and MARKS, H. P. Quart. J. Pharm. Pharmacol. 12(2) : 182-205. 1939.
3. PUGSLEY, L. I. J. Biol. Chem. 128 (Proc. Am. Soc. Biol. Chem. 33) : lxxx. 1939.

CONTINUED STUDIES ON CERCARIAL DERMATITIS AND THE TREMATODE FAMILY SCHISTOSOMATIDAE IN MANITOBA

PART I[1]

By J. A. McLeod[2] and G. E. Little[3]

Abstract

The life cycle of *Pseudobilharziella querquedulae* McLeod, 1937 has been completed experimentally and the relationship of the adult to *Cercaria physella* Talbot, 1936 has been definitely established. The female of the species and the eggs were recovered and are described for the first time. A report is given on the exposure of a number of species of molluscs to the miracidia of this schistosome and notes on the life cycle and adult stage of *Cercaria dermolestes* McLeod, 1940 are included. A case of hypersensitiveness in man to the proteins of schistosome cercariae is reported.

Introduction

The fact that "swimmers' itch" is caused by schistosome cercariae is well known at the present time to the majority of interested people. Each year sees additions made to the already long list of localities reporting outbreaks and also additional records of larvae capable of producing the disease. However, investigations of the biologies of the causative organisms have been singularly unproductive in the past 10 years and in many cases, instead of revealing the details of an organism's life cycle, an extensive investigation frequently has resulted only in the fortuitous discovery of new species of adult schistosomes. The work carried on in Manitoba by the senior author prior to the summer of 1940 is not an exception.

At the outset of the work reported herein, it was known that four species of schistosome cercariae, namely, *Cercaria elvae* (Miller, 1923) Talbot, 1936, *C. stagnicolae* Talbot, 1936, *C. physellae* Talbot, 1936, and *C. dermolestes* McLeod, 1940, were responsible for all the local outbreaks of cercarial dermatitis in man. It was also known that six species of adult schistosomes occur locally in water birds and that, while two of these are of rare occurrence, four species are quite common (3). Three of the more abundant species are found in herring gulls and ring-billed gulls and the fourth in blue-winged teal ducks.

Local ecological evidence of a rather circumstantial nature indicated that there was a possible connection between *C. stagnicolae* and either *Microbilharzia lari* McLeod, 1937 or *Ornithobilharzia aviani* McLeod, 1940, as all three occur in the Clear Lake area. The surveys indicated, however, that while the cercaria in Manitoba was confined to this region, the adult worms were not. Similarly, the ecological evidence indicated a possible connection

[1] *Manuscript received March 3, 1942.*
Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Man., with financial aid from the National Research Council of Canada.

[2] *Lecturer.*
[3] *Research Assistant.*

between either *C. elvae* or *C. dermolestes* and *Pseudobilharziella querquedulae* McLeod, 1937.

The senior author had previously reported the finding of adult *P. querquedulae* in immature teal ducks which were, as yet, unable to fly (op. cit.). These birds obviously had been hatched in the vicinity and were collected from a large slough where both *C. elvae* and *C. dermolestes* were very abundant. However, repeated experimental exposure of teal ducks to both species of cercariae failed to produce adult worms. There were no other bodies of water within reach of the infested birds and apparently other species of cercariae were absent from this habitat at the time the collection was made although they may have been present earlier in the season.

Brackett (1) has cited ecological evidence which strongly indicated a connection between *C. physellae* and *P. querquedulae*. The findings set down in this report not only substantiate but prove the accuracy of this assumption. Local studies of snail ecology have not been sufficiently detailed to be of more than superficial value in predicting the course of a schistosome's life cycle. The writers found in 1941 in at least one area under observation that the mature generation of the snail, *Physella gyrina* Say, died off just prior to or at about the same time as young teal made their way to the ponds.

On June 16th, a large lagoon at Delta, Man., was visited but no young teal were hatched as yet. However, mature *Physella gyrina* were abundant in the water and a few were giving off *C. physellae* in large numbers. The same area was visited 10 days later and the reverse condition was found. Freshly hatched teal were plentiful but adult *P. gyrina* had all disappeared and in their place were found many young specimens, none of which was liberating cercariae.

Such observations are inconclusive and only daily observations would determine if there was any overlapping and whether or not *C. physellae* and young ducks occurred simultaneously even for a few hours. Again, the possibility of infection occurring at a later date has not been ruled out and, as pointed out later in this paper, cercariae may emerge as early as 32 days after snail infection. Nothing is known regarding the length of time required for development of the parasite in the bird host but the finding of mature worms in young teal ducks referred to above in early August would lend credence to the theory that infection occurred in the bird during its first few days of life. Brackett's observations provide even stronger evidence in support of this belief.

The prospectus of the work, as in former investigations, centred around the experimental exposure of bird species known to be susceptible to schistosome infection to as many species as possible of local cercariae. In addition it was proposed to recover the eggs from the tissues of the primary host and to attempt to infect local snails with the miracidia.

Materials and Methods

In the experimental exposure of wild birds it is essential, of course, to employ only such specimens as have never come into contact with contaminated water prior to the beginning of the experiment. This necessitates the collecting and artificial incubation of the eggs or the obtaining of young specimens before they have left the nest. The nests of brooding ducks are readily located by adopting the method of having two workers drag a long rope between them over the brush and grass. Artificial incubation of fresh wild duck eggs in the laboratory usually results in a very poor hatch. However, if incubation has been carried on under natural conditions for 10 days or more it usually can be artificially brought to completion quite successfully. The writers' attempts to incubate eggs of the blue-winged teal in the laboratory have been rather unsuccessful and birds for experimental work were kindly supplied by the Delta Duck Station, Delta, Man.

Five young herring gulls were obtained in late June from nests on a barren reef in Grassy Narrows, Lake Winnipeg. While these birds ate readily in captivity when hand fed, care had to be exercised in arranging a diet high in calcium and vitamin D. Birds fed on meat scraps grew normally and were quite fat but in about four weeks they suddenly began to show signs of muscular weakness and lack of bone calcification. A change to a fish diet containing a good deal of bone and the addition of cod liver oil soon corrected the condition.

The locating of the females and eggs of *P. querquedulae* has been attempted by the writers several times in the past but without success. This was mainly due to faulty technique as the females seldom, if ever, occur in the mesenteric veins where the males are most abundant. Later, however, the fine veins under the gut serosa and even those of the submucosa were examined microscopically. This examination revealed moderate numbers of female worms but, because of their minute size, it was possible to recover intact only a small percentage. In the majority, it was necessary to dissect away the surrounding tissue. Ordinary dissecting needles or dental needles are much too coarse for this work but No. 00 insect pins fused into glass handles were found to be fairly satisfactory.

Due to the paucity of eggs in the faeces and the short time required for hatching it is almost impossible to recover the eggs of *P. querquedulae* in ordinary routine faecal examinations. A successful method suggested by Brackett involved splitting the gut and examining the inner surface by transmitted light under fairly high magnification. This technique revealed large numbers of eggs in clusters in the submucosa. They were recovered by dissecting them out individually or by simply scraping off the inner layers of tissue.

Fine, filamentous schistosomes appear to be very sensitive to slight changes in temperature, salt concentration, pH, and other factors, and almost invariably will coil into a tight knot if removed alive from the blood and placed in

any other medium. It was found impossible to relax them by shaking them in a test tube of physiological saline. The writers have found it advantageous to allow the worms to die in the vessels before removal is attempted, providing blood coagulation could be sufficiently retarded. It must be pointed out, however, that post-mortem contraction of the muscles makes recovery of small forms from the vessels of the gut wall very difficult.

The most satisfactory material was obtained from birds that had died during an epidemic of what appeared to be botulism poisoning. It appears that the toxin producing the disease has a paralysing effect on the worms. This suggests that the injection of certain drugs might be very helpful although it has not been tried.

Bouin's picro-formol solution was found to be the most satisfactory fixative for adult schistosomes. Mixtures of Delafield's haematoxylin and alum cochineal gave the best staining results. The proportion of each stain used depends to a great extent on the size of the worm. One part of haematoxylin to three parts of alum cochineal gives good results with large worms but the proportion of haematoxylin should be decreased as the size of the specimen diminishes.

Specimens Examined for Schistosomes

The incidence of snail infestation with schistosome larvae appears to vary enormously from year to year but, like many other organisms in nature, the larvae appear periodically to reach a maximum abundance with cyclic regularity. Even under physical conditions of near stability such as exist at Clear Lake there have been two pronounced outbreaks in the past 10 years, namely in 1933 and 1937, with intervals of comparative scarcity on either side.

While no definite data are available, general observations would lead one to believe that physical factors are not directly responsible for these variations. As regards biotic factors, whereas *Stagnicola emarginata* Sowerby, the snail host, varies considerably in abundance, there appears to be little or no correlation between these variations and the variations in the number of snail infestations. For example, *S. emarginata* was more abundant in Clear Lake in 1940 than during any year since this investigation was begun in 1933, yet infested specimens definitely were more rare. It appears that primary host abundance and the incidence of infestation of this animal species must constitute the variable to a great extent.

General observations and statistical data from former years have not been of great value because of pronounced variations in general conditions. The years prior to 1941 saw a steady and gradual reduction in the precipitation, a lowering of the water table in general, and a decided drop in the water level of marshes, ponds, and lakes. This continued until by early summer in 1940 all small lakes and ponds without sizable inflowing streams were dry and large marshes were very stagnant and much reduced in size. This resulted in a shift of the water bird population from the usual nesting ground to the north-

west and a disappearance of practically all the pond snails except a few of the hardier species. In 1941, heavy snow and rainfall restored these bodies of water to a near normal condition and birds and snails began to appear again. It is readily understood how such extreme conditions disrupt the normal course of events in the life cycles of animals requiring aquatic hosts and having free living aquatic stages.

The commonest representatives of the gastropod fauna of Manitoba had been examined previously for schistosome larvae but it was considered necessary to extend this phase of the work to increase the significance of the results. No complete records of the numbers of each species examined were kept but these ran into hundreds and in most cases thousands.

Species of snail examined	Species of cercaria recovered
Lymnaea stagnalis jugularis (Say).	*Cercaria elvae* (Talbot)
Stagnicola palustris elodes (Say)	*Cercaria dermolestes* (McLeod)
Stagnicola emarginata canadensis (Sowerby)	*Cercaria stagnicolae* (Talbot)
Physella gyrina (Say)	*Cercaria physellae* (Talbot)
Heliosoma trivolvis (Say)	
Gyraulus parvus (Say)	
Pseudosuccinea sp.	
Valvata tricarinata (Say)	
Amnicola sp.	
Amnicola sp.	

The above species comprise more than 90% of the snail population of the prairie lakes and sloughs west of the Precambrian portion of Manitoba. The first seven species are inhabitants of shallow water or occur above the water line but the last three are bottom forms found mainly in deep water and obtainable only by dredging.

Stagnicola emarginata in Manitoba, as in Michigan (McMullen (4)), is the one snail species found in abundance in clear, cold, weed-free water where bathing is most popular. The greatest percentage of cases of swimmers' itch are contracted at Clear Lake which is the habitat of *S. emarginata*. Occasional cases occur in bathers in Lake Winnipeg and Lake Manitoba simply because weedy bays are interspersed with stretches of open shore where the bathing is done. The vegetation is inhabited by *L. stagnalis* and *Stagnicola palustris* and wave motion is sufficient in some cases to carry the escaping cercariae into the water adjacent to the open beaches. People who have the temerity to bathe in sloughs or weedy patches on lake shores almost invariably contract swimmers' itch.

Physella gyrina appears to be only a moderately important factor in connection with dermatitis in Manitoba. This snail species is not rare by any means but rather is quite abundant in a definite type of habitat such as weedy

ponds and lagoons. However, such places are not extensively favoured as bathing places by the public. In addition, specimens of *P. gyrina* examined in the laboratory have shown a rather low incidence of infestation with schistosomes. During the past two summers large numbers have been examined and a relatively small number of infested specimens from the wild state were found. Such infested specimens as occurred were found mainly in the early summer or autumn. This is due to the fact that the mature generation largely dies off about the third week in June and not to any scarcity of adult schistosomes and their hosts.

Young herring gulls, domestic chickens, pigeons, and teal ducks were exposed separately to large numbers of *C. elvae*, *C. stagnicolae*, and *C. dermolestes*. With the exception of the pigeons all were negative when examined at post-mortem five weeks later. The pigeon exposed to *C. elvae* became sick on the 13th day following exposure and died on the 14th day. On examination no schistosomes or eggs were found but the liver was much enlarged, was abnormally pale, and extensively adhered to the body wall in the region of a large abscess on the left lobe. The specimen exposed to *C. dermolestes* was killed and examined on the 44th day and the fine veins of the intestinal wall contained a few mature specimens of a schistosome species.

What appears to be the same species of schistosome had been recovered a couple of weeks previously from the pectoral sandpiper (*Pisobia malanotos*) and the lesser yellow-legs (*Totanus flavipes*). The worms were seen fairly plainly through the wall of the vessels but because of their minute size only fragments were recovered. This material strongly indicates that they are members of the genus *Pseudobilharziella*, but is not adequate for the basis of a specific identification.

Prophylactic and Therapeutic Measures

As pointed out by McMullen (op. cit.) a number of nostrums have found their way on to the market in Michigan both as preventatives and as cures. In Manitoba, few, if any, so-called preventatives are offered for sale but a host of spurious cures are obtainable, none of which, incidentally, is of any value. McMullen suggests as a preventative the rapid drying of the skin with a coarse towel before the adhering water has had time to evaporate. As a palliative lotion he suggests calamine as the most effective and the most easily obtainable.

It has long been considered by the writers within the limits of possibility that the human system might become increasingly allergic to the proteins of schistosome larvae. Only one case has been followed up over a period of years but this one proves the belief to be not without foundation.

The senior author was moderately susceptible to schistosome attacks on first contact with the larvae eight years ago. This disease has been contracted while collecting snails each subsequent summer and the extent of the infestation varied from a few spots to fairly extensive areas. There appeared to be

little or no change in susceptibility but each succeeding attack was accompanied by greater discomfort. During the collecting of *Lymnaea stagnalis* and *Stagnicola palustris* in a lagoon off the Red River near Lockport in 1940, on one occasion an infection consisting of 92 papules was contracted on the right hand and lower six inches of the forearm. The usual itching was experienced for the first 24 hr. but at the end of that time the infected member began to swell and became highly inflamed. This continued for another 24 hr. during which the body temperature rose 1.5° F. and a sensation of general discomfort existed. Dark reddish to purple, torturous streaks appeared on the preaxial side from the wrist to the elbow and there was a marked enlargement and soreness of the supratrochlear and axillary lymph glands.

The infected area was then thoroughly scrubbed with hot water and soap solution and soaked for three ½-hour periods in hot Dakin's solution. Signs of septicemia and inflammation soon subsided and healing took place quickly. However, 10 days later numerous itchy lumps appeared on the skin in various parts of the body. These were reddish and inflamed but did not contain any trace of pus or bacterial infection. An expert dermatologist expressed the opinion that this represented an allergic reaction to the proteins of the foreign organism.

In later operations involving the hand picking of snails, the hand and arm were always given a good coating of solid vaseline. This gave complete protection even though the cercariae were quite abundant in the water.

Chemical control of snails or cercariae was beyond the scope of this present problem.

Descriptions

Pseudobilharziella querquedulae McLeod, 1937 (Fig. 1)

Generic diagnosis: *Pseudobilharziella* Ejsmont, 1929.

Male

Already described, McLeod (2).

Female

Filamentous worms of a creamy white colour, the body somewhat flattened dorsoventrally; almost uniform in width but tapering slightly toward the anterior end. The posterior end of the body is spatulate and in some specimens is expanded giving a suggestion of lobes. The cuticle is smooth or finely tuberculate. The oral sucker is terminal and cone-shaped, the mouth aperture, somewhat ventral in position. The acetabulum is pedunculate and circular in outline with a heavy rim. The oesophagus, a narrow tube, broadens abruptly into an expanded bulb just in front of the acetabulum; from it arise the caecal branches. The caecal branches unite just posterior to the ovary and the common caecum follows a torturous path backwards between the vitellaria to terminate blindly near the posterior end of the body. Its diameter is not uniform but where it comes close to the dorsal or ventral surfaces there is usually an irregular expansion.

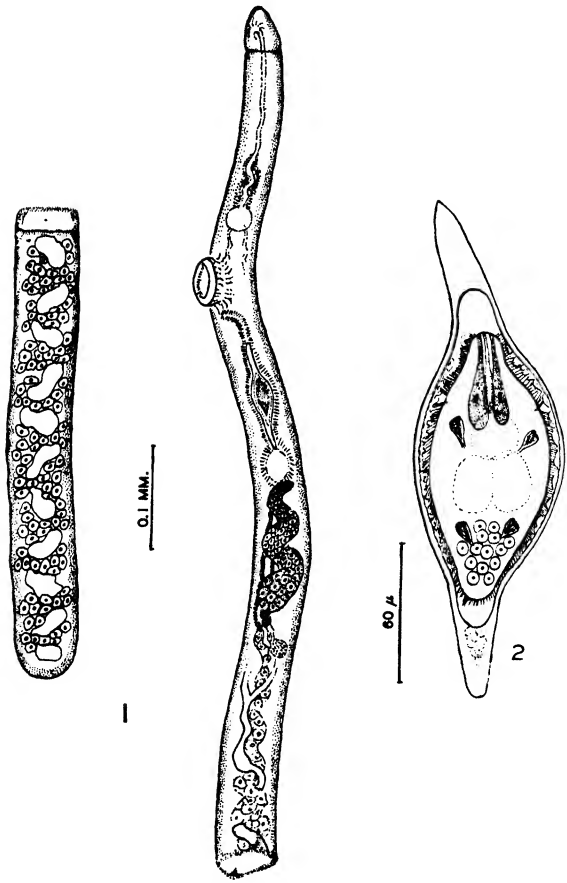


FIG. 1. *Pseudobilharziella querquedulae. Semidiagrammatic sketch of anterior and posterior portions of a stained and mounted female.* ×*150.*

FIG. 2. *Diagram of the egg and miracidium of Pseudobilharziella querquedula.* ×*350.*

The ovary is a stout, spiral, conical structure consisting of about three coils. The basal part of the cone is directed posteriad and the constituent cells are plainly visible. These appear to increase appreciably in size from the anterior to the posterior end. Laurer's canal was not observed to be present, but a well developed sac-like seminal receptacle is located just behind the ovary. Mehlis' gland is at the anterior end of the ovary and the genital pore is just behind the acetabulum. The uterus is moderately straight and contains not more than a single egg. The vitellaria consist of large clearly defined cells with distinct nuclei. These cells are not grouped into follicles but are irregularly dispersed in the region between the ovary and the posterior end. The vitelline cells appear to undergo disintegration as they pass anteriad since they

become granular in appearance, irregular in outline, and the nuclei become less definite as they approach the level of the ovary.

Measurements

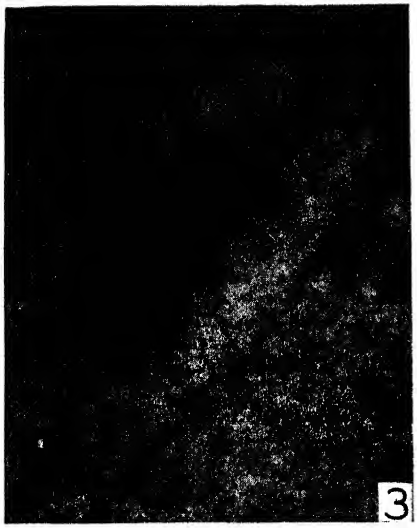
Body length 1.86 mm.; width 60 μ in broadest part and 40 μ at the anterior end; oral sucker 34 μ in maximum diameter; acetabulum located 250 μ from anterior end, 36 μ in diameter; ovary 170 μ behind the acetabulum, overall length, 120 μ (disregarding the total length of the loops), maximum width, 56 μ.

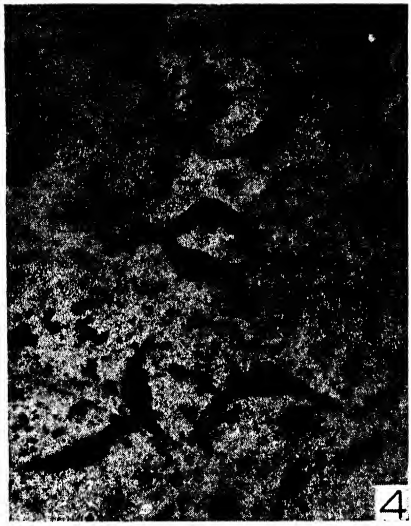
Egg (Figs. 2, 3, and 4)

The average length of 10 normal specimens removed from the intestinal submucosa was 217 μ and the width was 76 μ. The eggs apparently swell after deposition as those measured in the uterus showed an average length of only 140 μ and width of 30 μ. The eggs resemble those of *Bilharziella yokogawi* Oiso, 1927 in that there is a bulbous middle portion and the two ends are drawn out to considerable length and pointed (Fig. 2). The bulbous middle portion comprises approximately one-half the total length of the egg. Frequently the two ends are dissimilar, the anterior being crescent-shaped and sharply pointed while the posterior end is straight, conical, and bluntly pointed. The shell is very thin and fragile excepting the distal portions of the ends which appear to be solid. The large cavity contains a small amount of detritus adhering to the inside of the shell in addition to a well developed and very active miracidium.

The eggs are deposited in the blood vessels of the intestinal muscle layers and submucosa and usually are seen in loosely aggregated groups (Figs. 3 and 4). They occur throughout the length of the gut but are more abundant near the posterior end. Apparently they cause a minimum of damage to the tissue in working their way through to the lumen of the gut as no definite lesions from this cause have been seen. Apart from a slight sloughing of the inner layers of tissue, the gut wall appears to be in a healthy condition. One naturally expects to find lesions when eggs are present in quantity but in this case the absence of lesions cannot be accepted as proof that eggs are not present in the tissue of the intestinal wall.

A great variation in shape, general appearance, and viability of the eggs was observed. The one extreme was represented by a stout egg with a bulbous central portion, a thin, brownish shell, and a well developed miracidium, (Fig. 3). The tissue surrounding this type of egg showed no reaction or only a slight leucocyte infiltration. The other extreme was represented by a slender, crescent-shaped egg with finely attenuated ends and a small central cavity containing only a dark residue (Fig. 4). Each egg of this type was surrounded by a tough fibrous wall and could be removed only with great difficulty. When placed in water they failed to hatch or show any signs of viability. It is considered that these eggs have been embedded in the gut wall for a considerable time. Some bird hosts examined showed a high percentage of eggs of the latter type while in others they were rare or entirely absent.





FIGS. 3 AND 4. *Pseudobilharziella querquedulae. Surface view of a piece of cleared but unstained host intestine showing the eggs in situ. Taken by means of transmitted light using a Wratten green filter.*

FIG. 3. *Photomicrograph of young viable eggs.* ×*90.*

FIG. 4. *Photomicrograph of old inviable eggs.* ×*90.*

Normal eggs hatch in water in from 1 to 20 min. and the miracidium escapes through a longitudinal split in the bulbous part of the shell. The miracidium is of the usual type and is very active. Two pairs of flame cells are present and the nerve tissue is in the form of an indistinct, trilobed mass in the mid-body region.

Life Cycle

Miracidia in moderate numbers were placed in an aquarium containing snails as follows:

Species	Number exposed
Lymnaea stagnalis	10
Stagnicola palustris	11
Stagnicola emarginata	3
Heliosoma trivolvis	3
Physella gyrina	4
Physella sp.	9

These snails were not laboratory reared specimens but had been collected from their natural habitat and kept in the laboratory for a month prior to exposure. They had been checked for emerging cercariae twice before the beginning of the experiment and were rechecked daily thereafter. On the

32nd day following exposure, two specimens of *Physella gyrina* were found to be liberating large numbers of *Cercaria physellae* but all the others were still negative.

During the winter of 1940–41 several specimens of *Physella gyrina* were reared from the egg stage in the laboratory and in the following September four of these were exposed to the miracidia of *P. querquedulae*. Thirty-four days later three of them began liberating large numbers of cercariae. On close examination these proved to be typical specimens of *Cercaria physellae* Talbot, 1936 (5). The emergence of cercariae continued for approximately three months; then the infested snails died.

Discussion

It now remains only to expose teal ducks to *C. physellae* to complete the life cycle stages in the primary host. It has not been possible to do this during the past two years because of the inability to find or produce infested snails at the time when ducks were available. Extreme conditions such as the drying up of ponds and sloughs and the shifting of animal populations were mainly responsible for this. However, in view of the experimental results, both negative and positive, the writers feel they are correct in assuming that *C. physellae* is the larval stage of *P. querquedulae*. It has been demonstrated that *Q. discors* becomes infected with *P. querquedulae* in local waters but repeated exposures of specimens to all known species of local schistosomes cercariae excepting *C. physellae* have always given negative results. In the first exposure of snails to the miracidia it might be argued that the infection was picked up in nature. The results of exposure of laboratory reared specimens eliminates any possible error in this respect.

The examination of bird specimens also shows that *P. querquedulae* occurs fairly commonly in the pin-tail duck (*Dafila acuta*) and the spoon-billed duck (*Spatula clypeata*).

Investigations carried out by the writers so far have shed no light on the life cycle of either *C. elvae* or *C. stagnicolae* or the identity of the adult stages excepting that the cercariae would not infect herring gulls, chickens, pigeons, or teal ducks.

Experimental work did show, however, that *C. dermolestes* is the larval stage of a tiny species of *Pseudobilharziella* occurring naturally in shore birds such as the lesser yellow-legs (*Totanus flavipes*) and the pectoral sandpiper (*Pisobia malanotos*).

Acknowledgments

The writers desire to express their sincere appreciation to Prof. R. A. Wardle, University of Manitoba, for much kind advice and assistance. Thanks are given to Dr. Sterling Brackett, Faculty of Medicine, University of North Carolina, for his very kind co-operation at all times and to Dr. A. Hochbaum, Delta Duck Station, Delta, Man. Financial assistance from The National Research Council of Canada is gratefully acknowledged.

References

1. BRACKETT, S. Am. J. Hyg. D, 32(3) : 85-104. 1940.
2. McLEOD, J. A. J. Parasitol. 23(5) : 456-466. 1937.
3. McLEOD, J. A. Can. J. Research, D, 18(1) : 1-28. 1940.
4. McMULLEN, D. B. State of Michigan, Progress Rept., Div. of Water Itch Control, Stream Control Commission. 1939.
5. TALBOT, S. B. Am. J. Hyg. 23(2) : 372-384. 1936.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. JULY, 1942 NUMBER 7

NORTH PACIFIC POLYCHAETA, CHIEFLY FROM THE WEST COAST OF VANCOUVER ISLAND, ALASKA, AND BERING SEA[1]

BY E. BERKELEY[2] AND C. BERKELEY[2]

Abstract

A list is given of 175 species of Polychaeta collected at the northern extremity of the west coast of North America; seven of the species are new to the entire coast, 29 to the northern extremity, and four new to science.

Notes are given on distribution and taxonomic characters of many of the known forms and the new species are described.

Introduction

The following five sources have contributed to the material on which this report is based:—

1. A collection made by Mr. E. G. Hart on the west coast of Vancouver Island when he was serving as biologist on the Canadian Hydrographical Survey boat *William J. Stewart* in 1934.
2. A collection made by Prof. C. McLean Fraser on the west coast of the Queen Charlotte Islands in 1935.
3. A small littoral collection made at Pacofi Bay on the east coast of the Queen Charlotte Islands by Mr. P. Parizeau in 1940.
4. A collection made by Mr. W. Williams of the yacht *Stranger*, owned by Capt. F. E. Lewis, when that vessel was cruising off the coast of Alaska, in the Bering Sea, and in adjacent Arctic waters, in 1937.
5. A few miscellaneous specimens taken by various collectors during the past few years in the region covered by the report and submitted to the authors for examination.

It seems advisable to report on these five collections together since (with the exception of a few specimens taken by Mr. Williams north of Bering Strait, which fall more nearly within the arctic area) they all originate in the same geographical region and contain a large number of species of Polychaeta in common.

Mr. Hart's material, which constitutes the greatest part of the combined collections, was all taken between Barclay Sound and Tatchu Point on the

[1] *Manuscript received February 26, 1942.*
Contribution from the Pacific Biological Station, Nanaimo, B.C.
[2] *Voluntary Investigator.*

west coast of Vancouver Island. This stretch of coast was divided for purpose of record into four regions defined as follows:—

(*1*) Barclay Sound and the coast as far north as Amphitrite Point.
(*2*) Amphitrite Point to Estevan Point.
(*3*) Estevan Point to Bajo Point.
(*4*) Bajo Point to Tatchu Point.

The shore line and stations off the coast within lines running at right angles to the general direction of the coast line from the boundary points indicated are designated by the corresponding numbers in italics, (*1*), (*2*), (*3*), and (*4*), in recording Vancouver Island collecting localities in the report which follows. In addition to specimens taken between tide-marks, the collection comprises others taken both by trawl and by bottom sampler. The two latter classes are reported as "dredged" and the depth at which the specimens were taken is given for those in which it was recorded by the collector.

Prof. Fraser's collection was made on the west coast of the Queen Charlotte Islands between Houston-Stewart Channel and Rennell Sound. The majority of the specimens were taken between tide-marks, but a few taken by trawl, bottom sampler, or dredge are also in the collection. These also are reported as "dredged", but there is no record of depths. The precise locality at which each species was collected is given in the text.[1]

Mr. Williams' collection was submitted to the authors by Prof. G. E. MacGinitie, of the Kerckhoff Marine Laboratory, Corona del Mar, California. The data given by Mr. Williams of the conditions in which his specimens were taken is recorded.

Previous Work in the Region

Marenzeller (51) published a brief list of Polychaeta collected in Bering Sea by Dr. Krause in 1881. Moore (58, 59, 60) described the specimens collected by the U.S. Fisheries Steamer *Albatross* in 1903 at points throughout the region covered by the present communication. A number of specimens from Alaska are included in the report by Chamberlin (20) on the Polychaeta collected by the Canadian Arctic Expedition of 1913–18. Treadwell (73, 74) reported on the Polychaeta collected by Capt. R. A. Bartlett in Alaska in 1924. Bush (17) described tubicolous forms collected by the Harriman Alaska Expedition of 1899. A paper by the writers (10) contains an account of a few littoral species collected on the west coast of Vancouver Island in 1931. One of the writers has published a series of papers (4, 5, 6, 8, 9) dealing with the Polychaeta of the east coast of Vancouver Island. Although the polychaete fauna of this side of the island has characteristics differentiating it from that of the more exposed outer side and does not strictly come within the region defined as the subject of the present paper, it may be regarded as so doing in considering the wider aspects of the distribution of species. It will be so

[1] *Maps showing the regions covered by Mr. Hart's and Prof. Fraser's collections may be found in a paper by Dr. J. F. L. Hart on the reptant decapod Crustacea taken by the expeditions (Can. J. Research, 18(3): 86-105. 1940.).*

regarded in the following pages. In this paper are mentioned, therefore, some hitherto unpublished records of species taken by the writers off the east coast of Vancouver Island. Finally, there are a number of records of Polychaeta from the region under consideration scattered through the literature; reference to these will be made in the appropriate places in the text.

A large number of the species dealt with here will be found mentioned in the literature cited, but many of the records are based upon a single specimen or very few. It has been found possible to amplify the descriptions and to extend the range of distribution of several of them.

List of Species

The following is a complete list of the 175 species herein recorded. The species marked (*) are new records from the west coast of North America. Those marked (**) have been previously recorded from the coast, but only from the region south of that covered by the present report, or from the Arctic. *Syllis stewarti*, *Pista moorei*, *Pista pacifica*, and *Scionella estevanica* are listed as new species. The type specimen of each of these species is in the author's collection.

APHRODITIDAE

Aphrodite japonica Marenzeller
Aphrodite negligens Moore

POLYNOIDAE

Lepidonotus coelorus Moore
Malmgrenia nigralba Berkeley
Gattayana cirrosa (Pallas)
Eunoe nodosa (Sars)**
Harmothoe imbricata (Linné)
Evarnella triannulata (Moore)
Arctonoe vittata (Grube)
Arctonoe pulchra (Johnson)
Arctonoe fragilis (Baird)
Polyeunoa tuta (Grube)
Lagisca multisetosa Moore
Enipo cirrata Treadwell
Halosydna brevisetosa Kinberg
Lepidametria longicirrata (Berkeley)

SIGALIONIDAE

Sthenelais verruculosa Johnson
Pholoe minuta Fabricius

ACOETIDAE

Peisidice aspera Johnson

AMPHINOMIDAE

Euphrosyne hortensis Moore

PHYLLODOCIDAE

Phyllodoce madeirensis Langerhans**
Phyllodoce groenlandica (Oersted)
Paranaitis polynoides (Moore)
Eulalia bilineata Johnston
Eulalia viridis (Müller)
Eulalia nigrimaculata Moore
Notophyllum imbricatum Moore
Eteone spetsbergensis Malmgren var. *pacifica*
Eteone barbata (Malmgren)**

TOMOPTERIDAE

Tomopteris septentrionalis Apstein

SYLLIDAE

Syllis heterochaeta Moore
Syllis harti Berkeley
Syllis armillaris (Müller)
Syllis sclerolaema Ehlers
Syllis fasciata Malmgren
Syllis elongata (Johnson)
Syllis stewarti sp. n.
Trypanosyllis gemmipara Johnson
Odontosyllis phosphorea Moore var. *nanaimoensis* Berkeley
Odontosyllis phosphorea Moore
Sphaerosyllis pirifera Claparède
Sphaerosyllis hystrix Claparède
Exogone lourei Berkeley
Exogone verrugera (Claparède)
Autolytus prismaticus (Fabricius)

NEREIDAE

Nereis cyclurus Harrington
Nereis pelagica Linné
Nereis vexillosa Grube
Nereis eakini Hartman**
Nereis callaona (Grube)
Nereis virens (Sars)
Nereis dumerilii Audouin and Milne-Edwards var. *agassizi* Ehlers

HESIONIDAE

Kefersteinia cirrata (Keferstein)

NEPHTHYDIDAE

Nephthys caeca Fabricius
Nephthys longosetosa Oersted**
Nephthys californiensis Hartman**
Nephthys caecoides Hartman
Nephthys ciliata (Müller)
Nephthys punctata Hartman**

GLYCERIDAE

Hemipodia borealis Johnson
Glycera robusta Ehlers**
Glycera capitata Oersted
Glycera americana Leidy
Glycera gigantea Quatrefages**
Glycera nana Johnson
Glycinde armigera Moore
Glycinde picta Berkeley
Goniada brunnea Treadwell
Goniada maculata Oersted**

EUNICIDAE (LEODICIDAE)

Eunice biannulata Moore**
Eunice kobiensis McIntosh
Onuphis elegans (Johnson)
Onuphis iridescens (Johnson)
Onuphis conchylega Sars**
Diopatra ornata Moore
Lumbrinereis latreilli (Audouin and Milne-Edwards)
Lumbrinereis brevicirra Schmarda**
Lumbrinereis bifurcata McIntosh**
Lumbrinereis inflata Moore
Lumbrinereis similabris Treadwell
Arabella iricolor (Montagu)
Drilonereis filum Claparède**
Ninoe gemmea Moore**
Dorvillea moniloceras (Moore)

ARICIIDAE

Scoloplos armiger (O. F. Müller)
Nainereis laevigata (Grube)

SPIONIDAE

Laonice cirrata (Sars)
Polydora caeca (Oersted)
Prionospio pinnata Ehlers
Prionospio cirrifera Wiren

CHAETOPTERIDAE

Phyllochaetopterus prolifica Potts
Mesochaetopterus taylori Potts
Telepsavus costarum Claparède
Chaetopterus variopedatus (Renier)**

CIRRATULIDAE

Cirratulus robustus Johnson
Cirratulus cirratus (O. F. Müller)**
Caulleriella viridis (Langerhans) var. *pacifica* Berkeley
Tharyx multifilis Moore**
Chaetozone setosa Malmgren**
Dodecaceria pacifica (Fewkes)

CHLORAEMIDAE

Stylarioides arenosa (Webster)*
Stylarioides plumosa (O. F. Müller)
Stylarioides papillata (Johnson)

OPHELIIDAE

Ophelia limacina (Rathke)**
Travisia forbesii Johnston
Travisia brevis Moore
Travisia pupa Moore

CAPITELLIDAE

Notomastus pallidior Chamberlin**
Eisigella tenuis (Moore)

ARENICOLIDAE

Arenicola pusilla Quatrefages

MALDANIDAE

Praxilella affinis (Sars) var. *pacifica* Berkeley
Maldanella robusta Moore
Nicomache lumbricalis (Fabricius)
Maldane glebifex Grube*
Maldane similis Moore
Maldane sarsi Malmgren
Notoproctus pacificus (Moore)
Petaloproctus tenuis (Théel)*

AMMOCHARIDAE

Myriochele heeri Malmgren
Ammochares fusiformis (Delle Chiaje)

SABELLARIIDAE

Idanthyrsus armatus Kinberg
Sabellaria cementarium Moore

STERNASPIDAE

Sternaspis fossor Stimpson

AMPHICTENIDAE

Pectinaria belgica (Pallas)
Pectinaria auricoma (Müller)?
Cistenides brevicoma (Johnson)
Cistenides granulata Linné**
Cistenides hyperborea Malmgren

AMPHARETIDAE

Ampharete goesi Malmgren**
Ampharete arctica Malmgren
Ampharete eupalea Chamberlin
Ampharete grubei Malmgren**
Amphicteis scaphobranchiata Moore
Amphicteis mucronata Moore
Sabellides octocirrata Sars*
Amage anops (Johnson)
Melinna cristata (Sars)
Lysippe labiata Malmgren**
Samytha sexcirrata (Sars)

TEREBELLIDAE

Amphitrite cirrata (O. F. Müller)
Neoamphitrite robusta (Johnson)
Terebella ehrenbergi Grube*

Pista cristata (Müller)
Pista fratrella Chamberlin
Pista pacifica sp. n.
Pista moorei sp. n.
Thelepus crispus Johnson**
Polycirrus kerguelensis (McIntosh)*
Polycirrus caliendrum Claparède
Terebellides stroemi Sars
Scionella estevanica sp. n.
Neoleprea spiralis (Johnson)
Artacama conifera Moore**

SABELLIDAE

Demonax leucaspis Kinberg
Eudistylia vancouveri (Kinberg)
Eudistylia polymorpha (Johnson)
Distylia rugosa Moore
Potamilla torelli Malmgren*
Pseudopotamilla reniformis (Leuckart)
Branchiomma burrardum Berkeley
Schizobranchia nobilis Bush
Schizobranchia insignis Bush
Fabricia sabella (Ehrenberg)
Chone gracilis Moore
Chone infundibuliformis Kröyer
Euchone analis (Kröyer)

SERPULIDAE

Serpula vermicularis Linné
Salmacina dysteri (Huxley)** var. *tribranchiata* (Moore)
Crucigera irregularis Bush
Crucigera zygophera (Johnson)
Chitinopoma groenlandica Mörch
Spirorbis racemosus Pixell
Spirorbis medius Pixell
Spirorbis ambilateralis Pixell
Spirorbis spirillum (Linné)

Family APHRODITIDAE

Aphrodite japonica Marenzeller (50, p. 111; 60, p. 338; 37, p. 21 (with synonymy))

Occurrence. Vancouver Island (*2*), dredged in 130 metres, and (*3*), dredged in 75 to 90 metres.

Aphrodite negligens Moore (58, p. 526; 4, p. 211)

Occurrence. Vancouver Island (*4*), littoral and dredged in 45 metres.

Remarks. Moore found no asperities on the notosetae of this species and suggested that this might be due to wear. The present examples show well defined asperities on all the notosetae. More usually they occur in the posterior region of the body only.

Family POLYNOIDAE

Lepidonotus coelorus Moore (56, p. 412; 34, p. 108 (with synonymy))

Occurrence. Cleveland Passage, Alaska.

Remarks. The elytral papillae are as Moore describes, unlike the more pointed ones figured by Hartman (34, Fig. 35*d*) and found by the writers in specimens from California.

Malmgrenia nigralba Berkeley (4, p. 213)

Occurrence. Vancouver Island (*4*), dredged in 45 metres.

Gattayana cirrosa (Pallas) (24, p. 49; 60, p. 337)

Occurrence. Humpback Bay, Alaska, dredged in about 15 fathoms, and Cleveland Passage, Alaska.

Remarks. This species has also been collected in Departure Bay, east coast of Vancouver Island in 15 fathoms.

Eunoe nodosa (Sars) (24, p. 51)

Occurrence. Arctic Ocean, 68° 50′ N.; 169° 20′ W. Dredged in 30 fathoms.

Harmothoe imbricata (Linné) (24, p. 55; 4, p. 215)

Occurrence. Vancouver Island (*3*), dredged in 137 metres, and (*4*), littoral; Western Skidegate Narrows, Queen Charlotte Islands, littoral; Pacofi Bay, Queen Charlotte Islands, littoral; Cleveland Passage, Alaska.

Evarnella triannulata (Moore)

Harmothoe triannulata (62, p. 346), *Evarne triannulata* (4, p. 215)

Occurrence. Vancouver Island (*2*), dredged in about 555 metres.

Remarks. This species was originally dredged in considerable depths off the coast of southern California. Berkeley (4) records it from shallower waters off the east coast of Vancouver Island. It is interesting to note that in the more exposed waters of the outer coast of the island it is now again found in deep water. Chamberlin (19) points out that the generic name *Evarne* is preoccupied in Mollusca and substitutes *Evarnella*.

Arctonoe vittata (Grube) (34, p. 116; 37, p. 29 (with synonymy))

Occurrence. Vancouver Island (*2*), dredged in 77 and 172 metres, (*3*), dredged in 78 and 170 metres and littoral, and (*4*), littoral; Cleveland Passage, Alaska; Western Skidegate Narrows, Queen Charlotte Islands, littoral.

Arctonoe pulchra (Johnson) (34, p. 116)

Polynoe pulchra (41, p. 177), *Halosydna pulchra* (60, p. 329; 4, p. 212), *Lepidasthenia pulchra* (75, p. 144).

Occurrence. Vancouver Island (*2*), littoral, (*3*), dredged in 75 to 90 metres, and (*4*), dredged in 73 metres; Tassoo Harbour, Queen Charlotte Islands, dredged.

Remarks. Nearly all the specimens in this collection have the elytra mottled with brown, unlike those occurring on the east coast of Vancouver Island which usually have the pigment concentrated in a central area.

Arctonoe fragilis (Baird) (34, p. 116)

Polynoe fragilis (41, p. 179), *Halosydna fragilis* (4, p. 212), *Lepidasthenia fragilis* (75, p. 145).

Occurrence. Pacofi Bay, Queen Charlotte Islands, littoral.

Polyeunoa tuta (Grube)

Harmothoe tuta (42, p. 394), *Polynoe tuta* (60, p. 331; 4, p. 215)

Occurrence. Pacofi Bay, Queen Charlotte Islands, littoral.

Remarks. The elongated body with the elytra not confined to the anterior region justifies the classification of this species as above. A discussion of the genus *Polyeunoa* is given by Fauvel (28).

Lagisca multisetosa Moore (37, p. 54)

Occurrence. Vancouver Island (*2*), dredged in 45, 73, and 48.5 metres; Western Skidegate Narrows, Queen Charlotte Islands, littoral.

Enipo cirrata Treadwell (73, p. 1; 34, p. 120)

Occurrence. Pavlov Bay, Alaska.

Remarks. Commensal in the tube of *Nicomache lumbricalis* (Fabricius). Only the type specimen of this species has been known hitherto. This was also taken in Alaska. Hartman (34) has augmented the original description after re-examination of the type on which no elytra remain. The majority of the elytra are intact on the present specimen. They are small, suborbicular, smooth and glistening, have no marginal fringe, and are suffused with light brown pigment extending over about a third of the surface starting from the inner edge. They are well separated from one another leaving an area of the dorsum as wide as their diameter uncovered throughout the region of the body on which they occur. The distance between successive elytra varies from a complete diameter to a considerably smaller distance, but nowhere do they overlap. This separation of the elytra may be due in some measure to the flattened and extended condition of the specimen which is probably attributable to the close quarters in which it lives squeezed between the body and the tube of its maldanid host.

Halosydna brevisetosa Kinberg (37, p. 34 (with synonymy))

Occurrence. Vancouver Island (*2*), dredged in 77 metres and littoral, (*3*), littoral, and (*4*), littoral; Louscoone Bay and Skidegate Narrows, Queen Charlotte Islands, littoral.

Lepidametria longicirrata (Berkeley)

Lepidasthenia longicirrata (4, p. 214)

Occurrence. Vancouver Island (*4*), littoral.

Family Sigalionidae

Sthenelais verruculosa Johnson (41, p. 187; 37, p. 62)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Pholoe minuta Fabricius (24, p. 120; 4, p. 216)

Occurrence. Pavlov Bay, Alaska.

Family Acoetidae

Peisidice aspera Johnson (41, p. 184; 4, p. 216)

Occurrence. Vancouver Island (*3*), dredged in 55 metres.

Family Amphinomidae

Euphrosyne hortensis Moore (58, p. 534; 4, p. 212)

Occurrence. Cleveland Passage, Alaska.

Family Phyllodocidae

Phyllodoce (Anaitides) madeirensis Langerhans (24, p. 150; 15, p. 28)

Occurrence. Vancouver Island (*2*), dredged in about 555 metres.

Remarks. This species has been recorded by the authors from southern California (15). It was taken in that region in much shallower water. This and the present one seem to be the only records of the species from the west coast of North America.

Phyllodoce (Anaitides) groenlandica Oersted (24, p. 153; 5, p. 287)

Occurrence. Vancouver Island (*2*), dredged in 48.5 metres, and the following Alaska stations; Cleveland Passage, Nash Harbour, Nunivak Island (dredged in 8 to 10 fathoms), Spiriden Bay, Kodiak Island.

Paranaitis polynoides (Moore)

Anaitis polynoides (61, p. 339; 5, p. 287)

Occurrence. Tassoo Harbour, Queen Charlotte Islands, dredged.

Remarks. Southern (68) pointed out that the name *Anaitis* was preoccupied in Insecta.

Eulalia bilineata Johnston (24, p. 162; 5, p. 288)

Occurrence. Vancouver Island (*2*), littoral.

Eulalia viridis (Müller) (24, p. 160; 5, p. 288)

Occurrence. Vancouver Island (*2*), dredged in 73 metres, (*4*), littoral.

Eulalia nigrimaculata Moore (61, p. 344; 5, p. 288)

Genetyllis nigrimaculata (30, p. 119)

Occurrence. Vancouver Island (*3*), littoral.

Notophyllum imbricatum Moore (59, p. 217; 5, p. 287)

Occurrence. Vancouver Island (*4*), littoral.

Eteone spetsbergensis Malmgren var. *pacifica*

Eteone spetsbergensis (43, p. 102; 5, p. 289), *Eteone maculata* (72, p. 174 (non Oersted)), *Eteone pacifica* (30, p. 117)

Occurrence. Vancouver Island (*3*), littoral.

Remarks. This form agrees with the stem species except in having a spotted dorsum. In this particular and in all others it agrees with Treadwell's *E. maculata*. It is noteworthy that Treadwell's figure of the first parapodium of this species is inverted. Hartman (30) points out that the name *maculata* is preoccupied and suggests *pacifica* as a specific name. The writers regard it as no more than a colour variety of *E. spetsbergensis*.

Eteone (Mysta) barbata (Malmgren) (24, p. 176)

Occurrence. Arctic Ocean, 68° 37′ N.; 168° 53′ W. Dredged in 30 fathoms.

Family TOMOPTERIDAE

Tomopteris septentrionalis Apstein (24, p. 224; 5, p. 289)

Occurrence. Queen Charlotte Islands. Plankton.

Family SYLLIDAE

Syllis (Ehlersia) heterochaeta Moore (61, p. 322; 13, p. 42)

Occurrence. Vancouver Island (*2*), dredged in 45 metres.

Syllis harti Berkeley (13, p. 35)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Syllis armillaris (Müller) (24, p. 264; 4, p. 206)

Occurrence. Vancouver Island (*3*), and (*4*), littoral.

Syllis sclerolaema Ehlers (13, p. 40)

Occurrence. Vancouver Island (*4*), littoral.

Syllis fasciata Malmgren (49, p. 161; 15, p. 29)

Occurrence. Big Bay, Queen Charlotte Islands, littoral.

Remarks. This species is known from the east coast of Vancouver Island (authors' unpublished record) and has been reported (15) from Santa Cruz Island, southern California. Fauvel records it from Japan. It thus appears to be widely distributed in the North Pacific area.

Syllis elongata (Johnson) (4, p. 206; 13, p. 41)

Pionosyllis elongata (42, p. 403)

Occurrence. Vancouver Island (*3*), and (*4*), littoral.

Syllis stewarti sp. n.

Occurrence. Vancouver Island (*2*), littoral.

Description

A single specimen 115 mm. long and about 1.5 mm. wide. The body is uniformly slender and has no definite markings. The dorsum, as preserved, is a uniform light brown. It is arched, so that the inconspicuous parapodia seem to be almost ventral.

The prostomium is about three times as wide as long. There are four reddish eyes about equal in size, the anterior pair placed only slightly in advance of the posterior pair. The palps are fused basally for more than half their length. The tentacles and tentacular cirri are moniliform and all are about the same length. The median tentacle arises between the posterior pair of eyes. The dorsal cirri are also moniliform and those of the first few anterior parapodia are longer than the tentacles and tentacular cirri and have about 12 articles, but they soon become shorter and for the greater part of the body are not much longer than the length of the segments and have only seven to nine articles. The ventral cirri are slender and taper gradually. The setae resemble those of *S. spenceri* especially those figured in the description of that species (13). There is no sign of budding.

This may be no more than a variety of *S. spenceri*, though it is very much longer and has not the characteristic dorsal markings of that species. *S. spenceri* is known only in the sexually active form. It is possible that the present specimen represents the asexual form.

Trypanosyllis gemmipara Johnson (42, p. 405; 13, p. 42)

Occurrence. Vancouver Island (*4*), littoral.

Odontosyllis phosphorea Moore var. *nanaimoensis* Berkeley (4, p. 207)

Occurrence. Canoe Passage, Skidegate Channel, Queen Charlotte Islands. Dip-net collection.

Remarks. As would be anticipated from the method of collection, all the specimens are in the epitokous form.

Odontosyllis phosphorea Moore (61, p. 327)

Occurrence. Ucluelet, Vancouver Island (collected by Dr. J. L. Hart). From old pile below the low-tide-mark.

Remarks. This specimen is in the atokous form. The epitokous form of the species has not yet been taken in the region though that of the variety *nanaimoensis* is common.

Sphaerosyllis pirifera Claparède (24, p. 301; 13, p. 43)

Occurrence. Vancouver Island (*4*), littoral.

Sphaerosyllis hystrix Claparède (24, p. 301; 13 p. 43)

Occurrence. Vancouver Island (*4*), littoral.

Exogone lourei Berkeley (13, p. 44)

Occurrence. Vancouver Island (*4*), littoral.

Exogone verrugera (Claparède) (24, p. 307; 13, p. 44)

Occurrence. Vancouver Island (*4*), littoral.

Autolytus prismaticus (Fabricius) (20, p. 12B; 13, p. 48)

Occurrence. Vancouver Island (*4*), littoral.

Family Nereidae

Nereis (Cheilonereis) cyclurus Harrington (29, p. 214; 39, p. 219)

Occurrence. Vancouver Island (*2*), (*3*), (*4*). All dredged in about 73 metres.

Remarks. This species was originally described as a commensal with hermit crabs living in gastropod shells. Moore (60) records cases of apparently free-living forms and Berkeley (5) confirms the suggestion that the species is not necessarily a commensal. In the present collection only one of the five specimens was accompanied by a gastropod shell in which, pre-

sumably, it had lived. The other four specimens seem to have been free-living. It is noteworthy that these four specimens have spotted parapodia, as described previously for a single individual by one of the writers (5), whilst the commensal does not show this character.

Nereis pelagica Linné (24, p. 336; 39, p. 225)

Occurrence. Vancouver Island (*2*), dredged in about 555 metres; Skidegate Narrows, Queen Charlotte Islands, littoral; Cleveland Passage and Pavlov Bay, Alaska; Punuk Island, Bering Sea, dredged in 15 fathoms.

Remarks. The specimens from Pavlov Bay are in the heteronereid phase.

Nereis vexillosa Grube (21, p. 573; 5, p. 290)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), littoral; Masset Inlet, Queen Charlotte Islands (collector, Dr. A. Pritchard); Skidegate Narrows, Queen Charlotte Islands, littoral; Ketchikan and Humpback Bay, Alaska, littoral.

Nereis eakini Hartman (31, p. 472)

Occurrence. Vancouver Island (*4*), littoral; Big Bay and Skidegate Narrows, Queen Charlotte Islands, littoral.

Remarks. This species, previously recorded from California and Port Orchard, Washington, has also been taken by the authors on the east coast of Vancouver Island (unpublished record).

Nereis callaona (Grube) (23, p. 108; 39, p. 227 (with synonymy))

Occurrence. Vancouver Island (*3*), littoral.

Nereis virens (Sars) (24, p. 348; 5, p. 293)

Occurrence. Vancouver Island (*4*), littoral.

Platynereis dumerilii (Audouin & Milne-Edwards) var. *agassizi* Ehlers (54, p. 44)

Nereis agassizi (42, p. 399; 5, p. 292)

Occurrence. Tasoo Harbour, Queen Charlotte Islands, dredged; Skidegate Narrows, Queen Charlotte Islands, littoral.

Family HESIONIDAE

Kefersteinia cirrata (Keferstein) (24, p. 238)

Castalia fusca (44, p. 127; 4, p. 211)

Occurrence. Skidegate Narrows, Queen Charlotte Islands, littoral.

Remarks. This is a common shore form in the Nanaimo region, but, as it occurs there, it is small for the species. The present specimen is similar in all respects including that of size. McIntosh gives "tentacular cirri, two pairs". Actually there are eight pairs arranged in groups of 3, 3, and 2, as is described by Fauvel (assuming that the order of reading is from the setigers forward), but the two small, most ventral ones in the groups of three are very easily overlooked.

Family NEPHTHYDIDAE

Nephthys caeca Fabricius (24, p. 365; 5, p. 290)

Occurrence. Vancouver Island (*4*); Pavlov Bay, Nash Harbour, Nunivak Island, Bering Sea, Humpback Bay, Alaska, all littoral.

Nephthys longosetosa Oersted (24, p. 367).

Occurrence. Vancouver Island (*2*), dredged in 17 and 50 metres.

Remarks. This species occurs at Balboa, Lower California (authors' unpublished record). Monro (52) lists it from the Panama region. It has not been recorded elsewhere on the west coast of North America. It is not to be confused with *N. longisetosa* Ehlers which is, according to Hartman (33, p. 146), a synonym of *N. magellanica* Augener and is recorded by her under that name from southern California.

Nephthys californiensis Hartman (33, p. 150)

Occurrence. Vancouver Island (*3*), and (*4*), littoral.

Nephthys caecoides Hartman (33, p. 148)

Occurrence. Big Bay, Queen Charlotte Islands, dredged.

Nephthys ciliata (O. F. Müller) (24, p. 371; 60, p. 341; 5, p. 290)

Occurrence. Tassoo Harbour, Queen Charlotte Islands, dredged; Punuk Island, Bering Sea, dredged in 15 fathoms.

Nephthys punctata Hartman (33, p. 155)

Occurrence. Montagu Island, Alaska, dredged in 15 fathoms.

Remarks. This species has been taken off the east coast of Vancouver Island (authors' unpublished record).

Family GLYCERIDAE

Hemipodia borealis Johnson (42, p. 411; 39, p. 244)

Occurrence. Vancouver Island (*2*), dredged in 45 metres and littoral, (*4*), littoral.

Glycera robusta Ehlers (21, p. 656; 39, p. 246)

Occurrence. Vancouver Island (*3*), and (*4*), littoral; Houston Stewart Passage, Queen Charlotte Islands, littoral.

Remarks. These are the first records of this species north of California. One of the specimens is notable for the unusual development of the gill areas. These are strongly inflated and very prominent and occur on both the dorsal and ventral surfaces of the base of the foot. The writers recorded some very large specimens of the species from Elkhorn Slough, Monterey Bay, California, in 1935. Those from the Queen Charlotte Islands in the present collection are not as large as these, but are much larger than is usual.

Glycera capitata Oersted (24, p. 385; 6, p. 411)

Occurrence. Vancouver Island (*1*), dredged in 36 metres, (*2*), dredged in 172 metres, (*3*), dredged in 89 metres, and (*4*), littoral; Pavlov Bay, and Cleveland Passage, Alaska.

Glycera americana Leidy (21, p. 668; 39, p. 246 (with synonymy))

Occurrence. Vancouver Island (*2*) and (*3*), littoral, (*4*), littoral and dredged in 45 metres.

Glycera gigantea Quatrefages (24, p. 387)

Occurrence. Vancouver Island (*1*), dredged in 70 metres.

Remarks. The single specimen of this species seems to be a juvenile which, according to Fauvel, is the form described by Ehlers (21) as *G. lapidum.* No retractile branchiae can be made out. Ehlers says they are absent in *G. lapidum.* Monro (52) records *G. gigantea* from the Panama region and the writers (15) from southern California, but it does not seem to have been noted from more northerly latitudes.

Glycera nana Johnson (42, p. 411; 60, p. 347; 6, p. 411)

Occurrence. Vancouver Island (*2*), dredged in 17 metres, (*4*), dredged in 73 metres; Pacofi Bay, Queen Charlotte Islands, littoral; Cleveland Passage, Alaska.

Glycinde armigera Moore (63, p. 307; 39, p. 249 (*G. multidens*); 6, p. 411)

Occurrence. Vancouver Island (*1*), dredged in 36 metres, (*3*), dredged in 137 metres; Rennell Sound, Queen Charlotte Islands, dredged.

Glycinde picta Berkeley (6, p. 412)

Occurrence. Vancouver Island (*2*), dredged in 58 metres; Tassoo Harbour, Queen Charlotte Islands, dredged; Terror Bay, Kodiak Island, Alaska, Nash Harbour, Nunivak Island, Bering Sea, dredged in 8 to 10 fathoms, Cleveland Passage, Alaska.

Goniada brunnea Treadwell (70, p. 1174; 6, p. 412)

Occurrence. Vancouver Island (*1*), dredged in 36 metres.

Goniada maculata Oersted (24, p. 392; 39, p. 251)

Occurrence. Spiriden Bay, Kodiak Island, Alaska, dredged in 12 fathoms.

Family EUNICIDAE

Eunice biannulata Moore (57, p. 487; 38, p. 13)

Occurrence. Vancouver Island (*3*), littoral.

Remarks. Hartman (35, p. 96) gives the distribution of this species "from Alaska south to San Diego, California". The writers know of no previous record of it north of California except a tentative one (6, p. 407) from the Nanaimo region. They believe the present to be the first record of a typical specimen of the species north of California.

Eunice kobiensis McIntosh (43, p. 278; 60, p. 345)

Occurrence. Vancouver Island (*3*), littoral; Houston Stewart Passage and Skidegate Narrows, Queen Charlotte Islands, both littoral; Cleveland Passage, Alaska.

Onuphis elegans (Johnson) (11, p. 771; 15, p. 35)

Northia elegans (42, p. 406)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. The anal end of this species has not been described. It is usually absent from collected specimens. Several such ends are in this collection. They carry four almost equal, very long, thin anal cirri.

Onuphis iridescens (Johnson) (15, p. 36)

Northia iridescens (42, p. 408; 60, p. 345)

Occurrence. Vancouver Island (*2*), dredged in 101 metres, (*3*), dredged in 89, 58, and 137 metres, (*4*), dredged in 45 to 55 metres; Cleveland Passage, Alaska.

Onuphis conchylega Sars (24, p. 415)

Occurrence. Cleveland Passage, Alaska.

Diopatra ornata Moore (63, p. 273; 6, p. 408)

Occurrence. Vancouver Island (*2*), dredged in 18, 58, 60, 73, and 137 metres.

Remarks. Considerable variations occur in the characters on which the diagnosis of this species is based. The writers have discussed these and the differentiation of the species from *D. californica* Moore elsewhere (15, p. 36). The tubes of the species are very variable, being constructed, apparently, of whatever is available to the animal for the purpose, stuck together on a membranous foundation. In most of the specimens in this collection the material consisted of broken tubes of *Telepsavus costarum* Claparède.

Lumbrinereis latreilli (Audouin and Milne-Edwards) (24, p. 431; 15, p. 38)

Occurrence. Vancouver Island (*1*), dredged in 70 metres; Tassoo Harbour, Queen Charlotte Islands, dredged.

Remarks. This is the form erroneously recorded by Berkeley (6, p. 409) as *L. zonata* Johnson (= *L. brevicirra* Schmarda) from the Nanaimo region. Monro (54, p. 84) lists it from Galapagos and Gorgona Island and the writers have recently encountered it in material from southern California.

Lumbrinereis brevicirra Schmarda (37, p. 161 (with synonymy))

Occurrence. Vancouver Island (*2*), (*3*), and (*4*), all littoral.

Remarks. *L. brevicirra* does not seem to have been recorded previously from the coast north of California.

Lumbrinereis bifurcata McIntosh (43, p. 241; 71, p. 196)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Lumbrinereis inflata Moore (63, p. 289; 32, p. 32 (synonymy))

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Lumbrinereis similabris Treadwell (74, p. 5)

Occurrence. Vancouver Island (*2*), dredged in 45 metres; Terror Bay, Kodiak Island, Alaska; Teller, Alaska, dredged in five fathoms; Pavlov Bay, Alaska; McLeod Harbour, Montagu Island, Alaska, dredged in 15 fathoms; Spiriden Bay, Kodiak Island, dredged in 12 fathoms, Humpback Bay, Alaska, dredged in 15 fathoms.

Remarks. This species was originally described from Bering Sea. It comes near to *L. impatiens* Claparède. It differs markedly in the shape of the posterior lobe of the foot in anterior segments. It is very deep, almost semicircular, in outline. In the median region the posterior lobes are normal in shape, both lobes are about the same length, and they project only slightly. Towards the caudal end the whole parapodium projects well and both lobes are somewhat elongated, the posterior ones being the longer. The acicula are black. Simple crotchets start at the seventh setiger. Evidently the species is common in the Alaskan region. The writers have also encountered it in material from Cadboro Bay at the southern end of Vancouver Island.

Arabella iricolor (Montagu) (24, p. 438; 10, p. 313)

Occurrence. Vancouver Island (*2*), and (*3*), littoral.

Drilonereis filum Claparède (24, p. 436; 54, p. 88)

Occurrence. Humpback Bay, Alaska, dredged in about 15 fathoms.

Ninoe gemmea Moore (63, p. 283)

Occurrence. Vancouver Island (*2*), dredged in 172 metres, (*3*), dredged in 137 metres.

Remarks. This species seems to have been recorded only from California. The writers have, however, collected it from Comox Spit, on the east coast of Vancouver Island.

Dorvillea (Stauronereis) moniloceras (Moore) (61, p. 256; 6, p. 410)

Occurrence. Vancouver Island (*3*), and (*4*), littoral.

Family ARICIIDAE

Scoloplos armiger (O. F. Müller) (25, p. 20)

Occurrence. Cleveland Passage, Alaska.

Remarks. A specimen of this species was wrongly recorded by the writers, from the west coast of Vancouver Island (10) as *Scoloplos elongata* Johnson.

Nainereis laevigata (Grube) (25, p. 22; 10, p. 313)

Nainereis robusta (61, p. 262), *Nainereis longa* (61, p. 264; 6, p. 413)

Occurrence. Vancouver Island (*3*), littoral; Pacofi Bay, Queen Charlotte Islands.

Family SPIONIDAE

Laonice cirrata (Sars) (25, p. 38; 12, p. 474)

Occurrence. Vancouver Island (*2*), dredged in 130 metres, (*3*), dredged in 137 and 89 metres; Big Bay, Queen Charlotte Islands, dredged.

Polydora caeca (Oersted) (25, p. 52; 12, p. 469)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Remarks. This species is common in the walls of the tubes of *Nicomache lumbricalis* (Fabricius) (= *N. carinata* Moore) from this locality. It does not seem to have been recorded elsewhere on the west coast of North America.

Prionospio pinnata Ehlers (23, p. 163; 26, p. 173; 15, p. 42)

Paraprionospio tribranchiata (6, p. 415), *Prionospio alata* (64, p. 185)

Occurrence. Vancouver Island (*4*), dredged in 74 metres.

Prionospio cirrifera Wiren (25, p. 62; 26, p. 174)

Prionospio multibranchiata (6, p. 414)

Occurrence. Spiriden Bay, Kodiak Island, Alaska, dredged in 12 fathoms.

Family CHAETOPTERIDAE

Phyllochaetopterus prolifica Potts (67, p. 972; 8, p. 307)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), dredged in depths varying from 18 to 555 metres.

Remarks. This species is known to occur over a large area and at a wide range of depths off the east coast of Vancouver Island. Evidently it is even more abundant off the west coast.

Potts described in some detail the process of asexual reproduction of this species by autotomy and regeneration, but did not observe any individuals bearing genital products. He surmised that this was probably due to insufficient examination rather than that the asexual method of reproduction had entirely replaced the sexual. The writers' observations confirm this. Mature individuals of both sexes have frequently been found in the tubes. They differ in no essential respect from the commoner, asexually reproduced, forms except in the abdominal region. This is both longer and stouter than in the asexual forms. There are usually some 30 to 40 segments which are so distended with sexual products that the animal is removed from its tube only with difficulty. The sexes are readily distinguishable by the colour given to the abdominal region by the ova and sperms respectively. The former are bright red-brown, the latter white.

Mesochaetopterus taylori Potts (67, p. 958; 8, p. 307)

Occurrence. Vancouver Island (*2*), dredged in 50 metres (tube only), (*4*), dredged in 45 metres and littoral.

Telepsavus costarum Claparède (25, p. 82; 54, p. 1052)

Telepsavus sp. (67, p. 969), *Leptochaetopterus pottsi* (7, p. 441)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), dredged in depths varying from 45 to 90 metres, and (*4*), littoral.

Remarks. This species was originally described as having only two body regions, but Berkeley's (7) observation of the occurrence of a third region in material from the east coast of Vancouver Island led to Monro's (54) re-examination of Claparède's species from Naples, in which also he found the third region. The specimens from the west coast of Vancouver Island agree exactly with those from the east coast except that the great majority are very considerably smaller. Those on the east coast frequently measure some 50 cm. after fixation, those from the west coast usually no more than 5 cm. This is probably associated with the fact that in the former the tubes are widely separated in the beds, whilst in the latter they are densely crowded. The species evidently occurs over a large area and in considerable beds, in many cases the dredge samples consisting almost entirely of its tubes. It seems to occur only rarely as a littoral form on the west coast, but is common between tide-marks on the east coast.

The writers commented in 1932 on the masses of empty chaetopterid tubes thrown up by the tide at Long Bay, on the west coast of Vancouver Island, but at that time could not identify the species. It is now clear that they consisted of the ends of tubes of *T. costarum* which doubtless had been broken off by the heavy seas prevailing along the coast.

Chaetopterus variopedatus (Renier) (25, p. 77)

Occurrence. Cleveland Passage, Alaska.

Remarks. Four specimens are small, but otherwise quite typical.

Family CIRRATULIDAE

Cirratulus robustus Johnson (42, p. 423; 8, p. 308)

Occurrence. Vancouver Island (*3*), (*4*), littoral.

Cirratulus cirratus (O. F. Müller) (25, p. 94; 20, p. 20B (synonymy))

Occurrence. Ketchikan, Alaska, littoral.

Caulleriella viridis (Langerhans) var. *pacifica* Berkeley (8, p. 307)

Occurrence. Skidegate Narrows, Queen Charlotte Islands, littoral.

Tharyx multifilis Moore (61, p. 267)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Remarks. The variety *parva* of this species occurs on the east coast of Vancouver Island (8, p. 307), but this is the first record of the stem species north of California.

Chaetozone setosa Malmgren (25, p. 101)

Occurrence. Vancouver Island (*3*), dredged in 58 metres.

Remarks. The only previous record of this species from the coast is from Friday Harbour, Washington (76).

Dodecaceria pacifica (Fewkes) (61, p. 268; 10, p. 314)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), all littoral.

Family CHLORAEMIDAE

Stylarioides arenosa (Webster) (45, p. 98)

Occurrence. Vancouver Island (*4*), dredged in 45 metres.

Remarks. Both *S. eruca* Claparède and *S. capulata* Moore have bidentate ventral crotchets similar to those occurring in the present species, but in both of them the barring of the shafts of these setae is much wider than in *S. arenosa*. Fauvel (25, p. 119) regards the latter species as a synonym of *S. eruca*. However, *S. arenosa* appears to differ, not only in the respect mentioned, but also in the tendency toward grooving of the dorsum and in the heavy firm coating of sand (making it feel like a hard, sandy rod), which McIntosh describes and which is quite characteristic. For these reasons the writers are inclined to doubt the suggested synonymy.

Stylarioides plumosa (O. F. Müller) (25, p. 116; 9, p. 69; 15, p. 47)

Occurrence. Pacofi Bay, Queen Charlotte Islands; Spiriden Bay, Kodiak Island, Alaska, dredged in 12 fathoms; Cleveland Passage, Alaska.

Stylarioides papillata (Johnson) (9, p. 69; 15, p. 46)

Trophonia papillata (42, p. 416)

Occurrence. Skidegate Narrows, Queen Charlotte Islands, littoral.

Family Opheliidae

Ophelia limacina (Rathke) (25, p. 132; 35, p. 107)

Occurrence. Nash Harbour, Nunivak Island, Alaska, dredged in 8 to 10 fathoms; Cleveland Passage, Alaska.

Travisia forbesii Johnston (45, p. 26; 25, p. 138; 60, p. 354)

Occurrence. Nunivak Island, Bering Sea; Cleveland Passage, Alaska.

Travisia brevis Moore (64, p. 220; 8, p. 313)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres; Humpback Bay, Alaska, dredged in about 15 fathoms.

Travisia pupa Moore (59, p. 228; 8, p. 313)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Family Capitellidae

Notomastus pallidior Chamberlin (18, p. 179)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Remarks. The specimens agree with the somewhat incomplete description given by Chamberlin. They show slight prolongations of the neuropodia which may function as branchiae.

Eisigella tenuis (Moore)

Notomastus tenuis (61, p. 277; 8, p. 312)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. The absence of both branchiae and genital pores in this species necessitates the above generic reclassification.

Family Arenicolidae

Arenicola pusilla Quatrefages (3, p. 114; 10, p. 315)

Arenicola claparedii (9, p. 68)

Occurrence. Vancouver Island (*3*), littoral.

Family Maldanidae

Praxilella affinis (Sars) var. *pacifica* Berkeley (8, p. 313)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Remarks. This variety has a heavy hook in each of the first three setigers closely resembling that figured for *P. gracilis* by Arwidsson (1, Pl. 9, Fig. 303) rather than that of the stem species. This variation was overlooked in the original description.

Maldanella robusta Moore (59, p. 236; 8, p. 314)

Occurrence. Tassoo Harbour, Queen Charlotte Islands, dredged.

Nicomache lumbricalis (Fabricius) (25, p. 190)

Nicomache carinata (59, p. 242)

Occurrence. Vancouver Island (*2*), and (*3*), dredged in 130 and 137 metres, respectively. Pavlov Bay and Cleveland Passage, Alaska.

Remarks. The large number of specimens of this species in the collection has enabled the writers to study the variation in the characters by which Moore differentiates it from *N. carinata*. As a result it is found that the only character on which a separation might be based is the colour of the first six segments. In this the specimens agree with the description given by Moore for *N. carinata*, but this fact does not seem to justify specific separation.

The sandy tubes are thick-walled, brittle, and closely coiled with a smooth lining throughout. Arwidsson (1, p. 91) describes similar tubes for *N. lumbricalis*.

The spionid *Polydora caeca* was frequently present in the walls of the tubes.

Maldane glebifex Grube (25, p. 199)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Remarks. This species occurs widely on the east coast of Vancouver Island (authors' records), but has not been reported previously either there or elsewhere on the west coast of North America.

Maldane similis Moore (59, p. 233)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Remarks. The single specimen agrees closely with Moore's description except that it has one more chaetous segment. There are 19 such segments, segments 1 and 21 being achaetous.

Maldane sarsi Malmgren (25, p. 197; 60, p. 355; 64, p. 237)

Occurrence. Arctic ocean, 68° 37′ N.; 168° 53′ W., dredged in 30 fathoms.

Notoproctus pacificus (Moore) (2, p. 4; 8, p. 314)

Lumbriclymene pacifica (59, p. 246)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Petaloproctus tenuis (Théel) (1, p. 114)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres.

Remarks. The species has not been reported previously from the coast, but a specimen recorded by Berkeley (8, p. 314) from the Nanaimo region as *Petaloproctus* sp.? is almost certainly Arwidsson's variety *borealis* (1, p. 118).

Family AMMOCHARIDAE

Genus *Myriochele* Malmgren. char. emend.

As *Myriochele* except that eyes may or may not be present.

Myriochele heeri Malmgren (25, p. 204; 9, p. 67)

Occurrence. Vancouver Island (*3*), and (*4*), dredged in 89 and 74 metres, respectively.

Remarks. The specimens in this collection all have the line of small pigment spots across the dorsal side of the prostomium and the larger, more heavily pigmented, circular spots at either end of the line described by Berkeley (9) in specimens from the Nanaimo region. Further investigation has shown that undoubtedly these are eyes. In all other respects agreement with *Myriochele heeri* is complete. The presence of eyes does not justify specific separation, but it makes necessary the above modification of the definition of the genus since Malmgren gives "Pars cephalica oculis . . . . . destituta".

Ammochares fusiformis (Delle Chiaje) (9, p. 67)

Owenia fusiformis (25, p. 203), *Ammochares occidentalis* (42, p. 420)

Occurrence. Vancouver Island (*4*), dredged in 74 metres.

Family SABELLARIIDAE

Idanthyrsus armatus Kinberg (40, p. 90; 54, p. 1066)

Pallasia armata (23, p. 195), *Pallasia sexungula* (22, p. 125)

Occurrence. Vancouver Island (*2*), dredged in 77 metres, (*3*), and (*4*), littoral. Humpback Bay and Cleveland Passage, Alaska.

Remarks. This is the species recorded by Berkeley (9, p. 74) from the Nanaimo region and wrongly assigned to *Pallasia johnstoni* McIntosh.

Sabellaria cementarium Moore (59, p. 248; 15, p. 50)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), dredged in 50 to 90 metres.

Family STERNASPIDAE

Sternaspis fossor Stimpson (69, p. 29; 62, p. 144; 9, p. 69)

Occurrence. Vancouver Island (*3*), dredged in 137 to 180 metres. Arctic Ocean, 68° 37′ N.; 168° 53′ W., dredged in 30 fathoms.

Family AMPHICTENIDAE

Pectinaria belgica (Pallas) (25, p. 220; 15, p. 51)

Occurrence. Vancouver Island (*1*), dredged in 36 metres, (*3*), dredged in 137 metres. Rennell Sound, Queen Charlotte Islands, dredged. Terror Bay, Kodiak Island, Alaska.

Remarks. The specimens in this collection have the almost smooth edge to the dorsal plate and the characteristic caudal region which differentiate the species from *P. auricoma* (Müller).

Pectinaria auricoma (Müller)? (25, p. 222)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres.

Remarks. The single specimen is attributed as above with some doubt since, although the dorsal plate has the toothed edge which characterizes the species, the caudal region resembles that of *P. belgica* (Pallas).

Cistenides brevicoma (Johnson) (8, p. 311)

Pectinaria brevicoma (42, p. 423)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres.

Cistenides granulata (Linné) (48, p. 359; 20, p. 25B)

Occurrence. Point Hope, Alaska; Nash Harbour, Nunivak Island, Alaska, in 8 to 10 fathoms; Humpback Bay and Cleveland Passage, Alaska.

Remarks. The species is distinguished by the small number (seven or eight on each side) of blunt paleae, fewer than recorded for *C. brevicoma* (Johnson), which species it resembles in many respects. The uncini differ in that there are only three large teeth in the present species whilst those of *C. brevicoma* have four or five. The common occurrence of typical specimens of *C. granulata* in Alaskan waters seems to bear out the suggestion made by one of the writers (8, p. 311) that a transition occurs between these two species along the west coast of North America as the habitat becomes more southerly. If this be the case they should be regarded as one species.

Cistenides hyperborea Malmgren (48, p. 360; 71, p. 212)

Occurrence. Terror Bay, Kodiak Island, Alaska.

Remarks. This species has 11 to 12 paleae on each side. Those that are unworn have long, slender, pointed tips. The uncini have four large teeth. In this latter respect the specimen agrees with the Japanese form of the species as recorded by Nilsson (see 65, p. 57).

Family AMPHARETIDAE

Ampharete goesi Malmgren (48, p. 364)

Occurrence. Vancouver Island (*1*), dredged in 36 metres.

Remarks. The species is distinguished by the shape and number of the paleae and the arrangement of the branchiae in a straight line across the dorsum. It has not been recorded previously from the west coast of North America, but is a not uncommon arctic form.

Ampharete arctica Malmgren (48, p. 364; 15, p. 51)

Occurrence. Vancouver Island (*2*), (*3*), dredged in 50 and 95 metres, respectively.

Ampharete eupalea Chamberlin (20, p. 25B)

Ampharete seribranchiata (74, p. 7)

Occurrence. Arctic Ocean, 68° 37′ N.; 168° 53′ W., dredged in 30 fathoms.

Ampharete grubei Malmgren (48, p. 363)

Occurrence. Cleveland Passage, Alaska.

Amphicteis scaphobranchiata Moore (59, p. 255)

Occurrence. Vancouver Island (*1*), (*2*), (*3*), dredged in 36, 130, 137 metres, respectively.

Remarks. This species is probably synonymous with *A. japonica* McIntosh (47, p. 431). It occurs on the east coast of Vancouver Island (authors' unpublished record).

Amphicteis mucronata Moore (64, p. 203; 8, p. 310)

Occurrence. Vancouver Island (*3*), dredged in 137 to 180 metres. Tassoo Harbour, Queen Charlotte Islands, dredged.

Sabellides octocirrata Sars (25, p. 232)

Occurrence. Nash Harbour, Nunivak Island, and Spiriden Bay. Kodiak Island, Alaska, dredged in 8 to 12 fathoms.

Remarks. This species is found in fine mud tubes resembling those of *Myriochele heeri* Malmgren. It occurs on the east coast of Vancouver Island (authors' unpublished record).

Amage anops (Johnson) (15, p. 52)

Sabellides anops (42, p. 424)

Occurrence. Vancouver Island (*3*), dredged in 75 to 90 metres; Humpback Bay, Alaska, dredged in 15 fathoms.

Melinna cristata (Sars) (25, p. 237; 8, p. 311)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Lysippe labiata Malmgren (48, p. 367)

Occurrence. Cleveland Passage, Alaska.

Samytha sexcirrata (Sars) (48, p. 370; 20, p. 23B; 64, p. 214)

Occurrence. Cleveland Passage, Alaska.

Remarks. The single specimen agrees closely with the descriptions except that there are 20 abdominal segments, which is more than is recorded for the species. McIntosh (46, p. 78) gives "12 to 18". The number of these segments is somewhat variable in the genus.

Family TEREBELLIDAE

Amphitrite cirrata (O. F. Müller) (25, p. 251; 20, p. 22B; 15, p. 52)

Occurrence. McClinton Bay, Masset Inlet (collector, Dr. A. L. Pritchard); Pacofi Bay, Skidegate Narrows. All Queen Charlotte Islands, littoral.

Neoamphitrite robusta (Johnson) (36, p. 17; 75, p. 155)

Amphitrite robusta (42, p. 425), *Terebella robusta* (11, p. 773)

Occurrence. Vancouver Island (*2*), dredged in 555 metres, (*3*), dredged in 170 to 180 metres and littoral, (*4*), littoral. Louscoone Bay, Queen Charlotte Islands, littoral. Ketchikan, Alaska, littoral.

Remarks. The example collected at the greatest depth (555 metres) has a hard tube constructed of coarse sand, quite unlike the muddy tube in which the species is found between tide-marks, but no difference can be observed in the technical characters of the animal.

Terebella ehrenbergi Grube (26, p. 226; 27, p. 80)

Occurrence. Vancouver Island (*3*), littoral.

Remarks. The majority of records of this species are from warmer seas. It has not been recorded previously from the west coast of North America. It appears to be fairly common in Japan and has been found recently associated with introduced Japanese oysters at Ladysmith Harbour on the east coast of Vancouver Island. It may well have reached the coast via Japan.

Pista cristata (Müller) (25, p. 266; 60, p. 351; 15, p. 53)

Occurrence. Vancouver Island (*3*), dredged in 137 metres; Spiriden Bay, Kodiak Island, Alaska.

Pista fratrella Chamberlin (19, p. 18; 8, p. 309; 15, p. 53)

Occurrence. Vancouver Island (*2*), dredged in 130 and 172 metres, (*3*), dredged in 75 to 90 metres; off Cape Cook, west coast of Vancouver Island in a deep net haul (collector, Dr. H. C. Williamson).

Pista pacifica sp. n.

Pista elongata (11, p. 773 (non Moore))

Occurrence. Vancouver Island (*4*), littoral.

Remarks. Four anterior portions of this species are in the collection together with a number of empty tubes. The characters are exactly those of the forms from Elkhorn Slough, California, described by the writers under the name *P. elongata* Moore. The writers pointed out wherein they differed from this latter species, but regarded the differences as coming within the limits of variation. Examination of the present specimens convinces them that this is not so. The outstanding differences are the presence of a definite tongue-shaped wing on IV (Fig. 1), which is entirely absent in *P. elongata*, and the form of the long-stemmed thoracic uncinus (Fig. 2).

The tubes of the two species are quite different. That of *P. elongata* has been described by one of the writers (8, p. 309). That of the present species has a heavy coating of mud and sand and terminates in a hood bent at an angle to the tube and ornamented round the mouth with long pendant processes (Fig. 3).

P. macrolobata Hessle has lobes on the anterior segments very similar to those of this species, but differs in having only two pairs of branchiae and in the form of the long-stemmed thoracic uncinus.

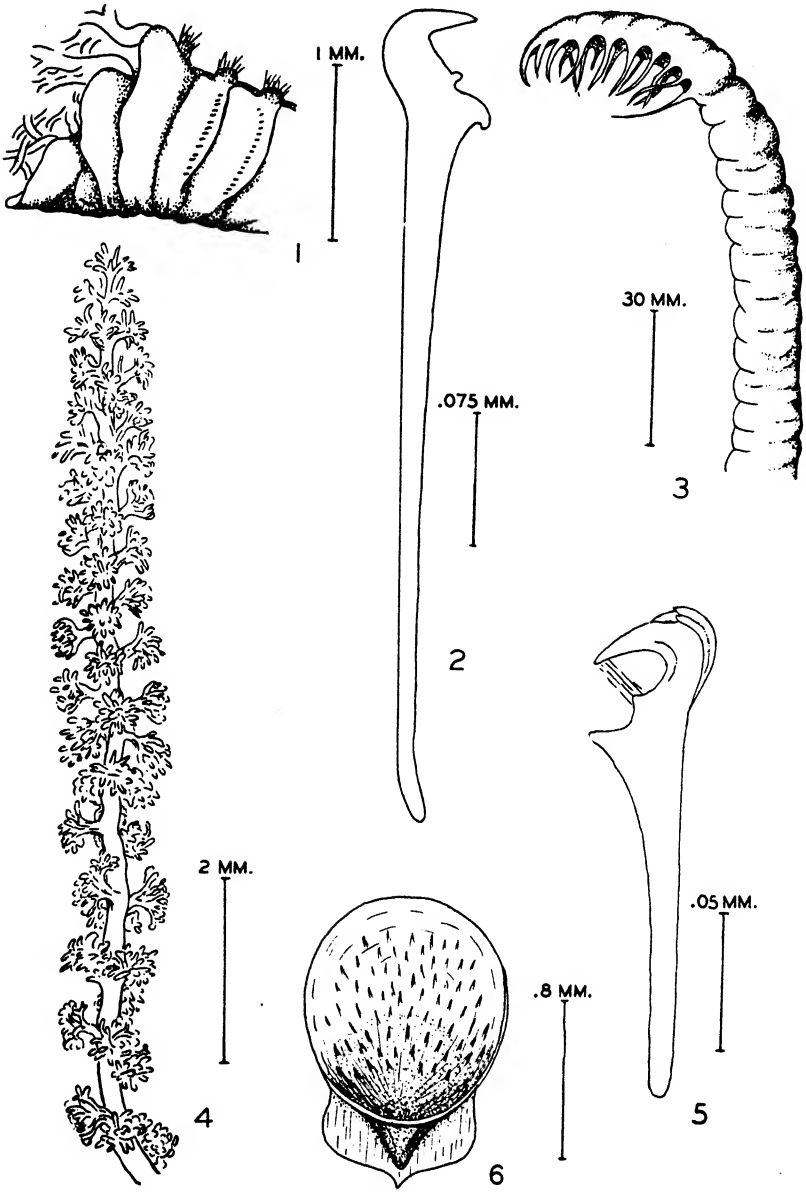


FIG. 1. *Pista pacifica sp. n. Anterior end.* FIG. 2. *Pista pacifica sp. n. Thoracic uncinus.* FIG. 3. *Pista pacifica sp. n. Tube.* FIG. 4. *Pista moorei sp. n. Branchia.* FIG. 5. *Pista moorei sp. n. Thoracic uncinus.* FIG. 6. *Spirorbis medius Pixell. Opercular plate newly regenerated.*

Pista moorei sp. n.

Pista sp.? (64, p. 197)

Occurrence. Vancouver Island (*1*), dredged in 36 metres.

Remarks. The single example is almost certainly identical with the species described from southern California by Moore, but left unnamed by him because his material was imperfect. The writers have examined other specimens from the east coast of Vancouver Island and from Monterey Bay, California, and base this opinion on a consideration of all the material. The gills and the wings on the anterior segments correspond with Moore's description and the former are unlike those of any other species of *Pista* known to the writers. When fully expanded they are long and trailing and have whorls of branchlets at intervals throughout their length (Fig. 4). The wings on I and II are generally as Moore describes, but the development of those on III varies considerably in different specimens. They are always large and extend from the ventral side to above the dorsum, but the tongue-like extension may rise only a little above the dorsum or so much above it as to reach beyond the prostomium when directed forward, as Moore describes. The wings on IV correspond with Moore's description, but are not placed as far ventrally as those on II in the writers' specimens.

An uncinus from the first thoracic torus is shown in Fig. 5.

Thelepus crispus Johnson (42, p. 428; 61, p. 275)

Occurrence. Vancouver Island (*2*), and (*4*), littoral. Pacofi Bay and Skidegate Narrows, Queen Charlotte Islands, littoral.

Remarks. This species is similar to *T. setosus* (Quatrefages) and the writers have (14) expressed the opinion that they were indistinguishable. The material in this collection shows this opinion to have been erroneous. The two species are most readily differentiated by the arrangement of the uncini. Excepting on the first few somites these form flattened rings in *T. crispus*, whilst they remain single lines throughout the body in *T. setosus*. Other points of difference are:—

1. The shape of the uncinigerous pinnules, which are wart-like and not very prominent in *T. crispus*, square and projecting in *T. setosus*.

2. The extension of the notopods, which persist until near the posterior extremity in *T. crispus*, but cease many segments short of it in *T. setosus*.

3. The definition of the ventral plates. These are fairly well defined in *T. setosus*, but are so much furrowed and folded in *T. crispus* as to be almost indistinguishable. The species has not been recorded previously north of California, but it has been taken at Port Townsend, Washington (collector, Mr. E. F. Ricketts; authors' identification).

Polycirrus kerguelensis (McIntosh) (53, p. 194)

Ereutho kerguelensis (43, p. 474)

Occurrence. Vancouver Island (*2*), dredged in 58 metres.

Remarks. A single complete example agrees exactly with the form described by McIntosh as *Ereutho kerguelensis* except in that it has 12 thoracic notopodia, whilst McIntosh gives 13. Monro (55, p. 184) finds that the number may vary from 11 to 15.

This form is to be distinguished from that described by McIntosh (43, p. 475) as *Polycirrus kerguelensis*, which has a different form of uncinus and agrees with *Ereutho antarctica* Willey (16, p. 95).

Polycirrus caliendrum Claparède (25, p. 281)

Polycirrus perplexus (64, p. 198; 8, p. 310)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. There seems to be nothing to differentiate this form from Moore's *P. perplexus*. The form of the uncinus is very variable and no valid distinction can be based upon it. Fauvel points out that *P. caliendrum* is very similar to, if not identical with *P. nervosus* Marenzeller, which is recorded from Japan (27, p. 83).

Terrebellides stroemi Sars (25, p. 291; 60, p. 352; 8, p. 310; 20, p. 23B)

Occurrence. Vancouver Island (*3*), dredged in 89 to 180 metres. Spiriden Bay, Kodiak Island, Alaska. Nash Harbour, Nunivak Island, Alaska, dredged in 8 to 10 fathoms.

Scionella estevanica sp. n.

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Description. Two specimens, of which the posterior region is incomplete, occur in the collection. The body is long and not more than 1 mm. wide, considerably more slender than *Scionella japonica* Moore (56, p. 473), which appears to be the only other known representative of the genus. It resembles the latter species closely in all other morphological characters except the following:

1. There is a long low ridge on the dorsum of IV instead of the shorter high fold of *S. japonica*.
2. The lateral lobes of the first four somites do not rise successively as abruptly as in *S. japonica*, so that those of IV do not attain as dorsal a position.
3. The single pair of branchiae is attached to the anterior edge of II instead of to the fold on IV as in *S. japonica*.
4. The branchiae are bottle-brush shaped and have about 20 densely packed whorls.

The tubes are thin and membranous and are beset with sand grains. They fit the animal very tightly.

Scionella japonica has been recorded from Friday Harbour, Washington (76) and occurs on the east coast of Vancouver Island (authors' unpublished record).

Neoleprea spiralis (Johnson) (36, p. 17)

Amphitrite spiralis (42, p. 426; 8, p. 308)

Occurrence. Vancouver Island (*2*), littoral.

Artacama conifera Moore (58, p. 853; 64, p. 191)

Occurrence. Nootka Sound, west coast of Vancouver Island (collector, J. P. Tully).

Remarks. This species has been recorded from Friday Harbour, Washington (76) and has also been taken off the east coast of Vancouver Island (authors' unpublished record).

Family SABELLIDAE

Demonax leucaspis Kinberg (54, p. 1075 (with synonymy); 15, p. 54)

Occurrence. On an old pile below low-water-mark at Ucluelet, west coast Vancouver Island (collector, Dr. J. L. Hart).

Eudistylia vancouveri (Kinberg) (36, p. 22)

Eudistylia gigantea (17, p. 210; 9, p. 70; 10, p. 315)

Occurrence. Vancouver Island (*4*), littoral.

Eudistylia polymorpha (Johnson) (36, p. 23)

Bispira polymorpha (42, p. 428; 9, p. 70)

Occurrence. Vancouver Island (*2*), dredged in about 555 metres.

Distylia rugosa Moore (57, p. 499; 61, p. 289; 9, p. 69)

Occurrence. Vancouver Island (*2*), littoral.

Potamilla torelli Malmgren (25, p. 310)

Occurrence. Vancouver Island (*3*), littoral.

Remarks. This species is distinguished by the absence of branchial eyes. Two specimens, apparently mature, occur in the collection and correspond closely with Fauvel's description except that no pigment remains. Several younger forms, differing by having fewer branchial filaments (sometimes as few as three on each side) and very pointed ends to the collar, also occur. All were found in fragile tubes embedded in the walls of the tubes of *Idanthyrsus armatus* Kinberg.

Pseudopotamilla reniformis (Leuckhart) (25, p. 309)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. This species, widely distributed in Arctic seas and elsewhere, has been recorded from Alaska (60, p. 359) and from Vancouver Island (9, p. 70; 10, p. 315). Hartman (36, p. 27) expresses doubt as to the validity of these records in view of the possible confusion of this species with *P. intermedia* Moore. The writers are inclined to regard the latter as a synonym, the only significant difference being that whilst *P. intermedia* is said to have never more than two rather inconspicuous eyes, and sometimes only one or none, on a radiole, *P. reniformis*, as described by Fauvel, may have as many as eight large conspicuous ones. However, other authors have failed to observe more than two eyes. This latter condition holds in the specimens in the present collection. They have eight thoracic setigers, resembling in this and other respects the specimens previously described from Vancouver Island. Fauvel gives 9 to 12, Johansson (40, p. 143) seven to nine. It is possible that the species referred to by Hartman as *P. reniformis* (Montagu) is a different form since she describes it as a "warm water species".

Branchiomma burrardum Berkeley (9, p. 71; 15, p. 55)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres.

Schizobranchia nobilis Bush (17, p. 207; 9, p. 71)

Occurrence. Vancouver Island (*3*), littoral. Pavlov Bay, Alaska.

Schizobranchia insignis Bush (17, p. 206; 9, p. 70)

Occurrence. Vancouver Island (*4*), littoral. Pavlov Bay and Cleveland Passage, Alaska.

Remarks. The writers are very doubtful whether this and the preceding species should be separated. The characters on which differentiation has been based are very variable and the two forms are found together.

Fabricia sabella (Ehrenberg) (25, p. 325; 9, p. 73)

Occurrence. Vancouver Island (*4*), littoral.

Chone gracilis Moore (59, p. 257; 10, p. 315)

Occurrence. Pavlov Bay, Alaska.

Chone infundibuliformis Kröyer (25, p. 334; 15, p. 55)

Occurrence. Vancouver Island (*4*), littoral; Nash Harbour, Nunivak Island, Alaska, dredged in 8 to 10 fathoms; Pavlov Bay, Alaska.

Euchone analis (Kröyer) (20, p. 27B; 9, p. 71)

Occurrence. Cleveland Passage, Alaska.

Family SERPULIDAE

Serpula vermicularis Linné (25, p. 351; 9, p. 73; 47, p. 353)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), all littoral.

Salmacina dysteri (Huxley) var. *tribranchiata* (Moore) (54, p. 1090; 15, p. 56)

Occurrence. Vancouver Island (*4*), littoral.

Crucigera irregularis Bush (66, p. 786; 9, p. 73)

Occurrence. Vancouver Island (*2*), littoral and dredged in 172 metres, (*3*), dredged in 113 and 155 metres. Humpback Bay, Alaska, dredged in about 15 fathoms.

Crucigera zygophera (Johnson) (66, p. 786; 9, p. 73)

Serpula zygophera (42, p. 433)

Occurrence. Vancouver Island (*2*), dredged in 48 metres.

Chitinopoma groenlandica Mörch (66, p. 790; 9, p. 74)

Occurrence. Vancouver Island (*2*), dredged in 64, 70, and 130 metres.

Spirorbis racemosus Pixell (66, p. 799)

Occurrence. Vancouver Island (*1*), littoral.

Remarks. This species does not appear to have been recorded since it was originally described from the east coast of Vancouver Island. It is noteworthy that the present specimens were collected between tide-marks whereas the original ones were dredged in 15 to 25 fathoms.

Spirorbis medius Pixell (66, p. 800; 68, p. 149; 25, p. 398)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. A number of representatives of this species in the collection have no notch in the serrated edge of the collar setae. Others have only one or two setae notched, the remainder with unbroken edge. This is in agreement with Southern's findings in Irish specimens. The calcareous plate of the operculum can be regenerated. Pixell's figure represents a worn plate. The newly regenerated plate is densely covered with sharp conical transparent spines and the wings are wider than they are when it is worn (Fig. 6).

Spirorbis ambilateralis Pixell (66, p. 798; 9, p. 74)

Occurrence. Vancouver Island (*2*), littoral.

Spirorbis spirillum (Linné) (25, p. 392; 66, p. 796; 9, p. 74)

Occurrence. Vancouver Island (*3*), littoral.

References

1. Arwidsson, I. Studien über die skandinavischen und arktischen Maldaniden. Upsala. 1906.
2. Arwidsson, I. Kgl. Svenska Vetenskapsakad. Handl. 63(7) : 1-46. 1922.
3. Ashworth, J. H. Cat. of Chaetopoda of Brit. Mus. Polychaeta. Pt. I. 1912.
4. Berkeley, E. Contrib. Can. Biol. (n.s.) 1(11) : 203-218. 1923.
5. Berkeley, E. Contrib. Can. Biol. (n.s.) 2(12) : 285-293. 1924.
6. Berkeley, E. Contrib. Can. Biol. Fisheries (n.s.), 3(17) : 405-422. 1927.
7. Berkeley, E. Proc. Zool. Soc. London, 1927 : 441-445. 1927.
8. Berkeley, E. Contrib. Can. Biol. Fisheries (n.s.), 4(22) : 305-316. 1929.
9. Berkeley, E. Contrib. Can. Biol. Fisheries (n.s.), 6(5) : 65-77. 1930.
10. Berkeley, E. and Berkeley, C. Contrib. Can. Biol. Fisheries (n.s.), 7(24) : 309-318. 1932.
11. Berkeley, E. and Berkeley, C. Am. Midland Nat. 16(5) : 766-775. 1935.
12. Berkeley, E. and Berkeley, C. Ann. Mag. Nat. Hist. (Ser. 10) 18 : 468-477. 1936.
13. Berkeley, E. and Berkeley, C. Ann. Mag. Nat. Hist. (Ser. 11) 1 : 33-49. 1938.
14. Berkeley, E. and Berkeley, C. Ann. Mag. Nat. Hist. (Ser. 11) 3 : 321-346. 1939.
15. Berkeley, E. and Berkeley, C. Bull. S. Calif. Acad. Sci. 40 : 16-60. 1941.
16. Benham, W. B. Sci. Rept. Australasian Antarctic Exped. 6, Pt. 3. 1921.
17. Bush, K. J. Harriman Alaska Exped. 12 : 169-355. 1904.
18. Chamberlin, R. V. Proc. Biol. Soc. Wash. 31 : 173-180. 1918.
19. Chamberlin, R. V. Mem. Museum Comp. Zool. Harvard, 48 : 1-514. 1919.
20. Chamberlin, R. V. Rept. Can. Arctic Exped. 9(B) : 1-41. 1920.
21. Ehlers, E. Die Borstenwürmer. Leipzig. 1868.
22. Ehlers, E. Hamburger Magalhaenische Sammelreise. 1897.
23. Ehlers, E. Festschr. Kgl. Ges. wiss. Göttingen. 1901.

24. FAUVEL, P. Faune de France, 5 : 1-488. 1923.
25. FAUVEL, P. Faune de France, 16 : 1-494. 1927.
26. FAUVEL, P. Mem. Indian Museum, 12(1) : 1-232. 1932.
27. FAUVEL, P. Mem. Coll. Sci., Kyoto Imp. Univ. B, 12(1) : 41-92. 1936.
28. FAUVEL, P. Rés. Voyage *Belgica*, 1897–99. 1936.
29. HARRINGTON, N. R. Trans. New York Acad. Sci. 16 : 214-221. 1897.
30. HARTMAN, O. Univ. Calif. Pub. Zool. 41 : 117-132. 1936.
31. HARTMAN, O. Proc. U.S. Natl. Museum, 83 : 467-480. 1936.
32. HARTMAN, O. J. Wash. Acad. Sci. 26 : 31-32. 1936.
33. HARTMAN, O. Proc. U.S. Natl. Museum, 85 : 143-158. 1938.
34. HARTMAN, O. Proc. U.S. Natl. Museum, 86 : 107-134. 1938.
35. HARTMAN, O. Univ. Calif. Pub. Zool. 43 : 93-111. 1938.
36. HARTMAN, O. Bull. Museum Comp. Zool. Harvard, 85(1) : 1-31. 1938.
37. HARTMAN, O. Allan Hancock Pacific Exped. 7, No. 1. 1939.
38. HARTMAN, O. Smithsonian Inst. Pub. Misc. Collections, 98. 1939.
39. HARTMAN, O. Allan Hancock Pacific Exped. 7, No. 3. 1940.
40. JOHANSSON, K. E. Zool. Bidrag Upsala, 2. 1927.
41. JOHNSON, H. P. Proc. Calif. Acad. Sci. (Ser. 3) 1(5) : 153-198. 1897.
42. JOHNSON, H. P. Proc. Boston Soc. Nat. Hist. 29(18) : 381-437. 1901.
43. MCINTOSH, W. C. Rept. Sci. Results Voyage *H.M.S. Challenger*, Zool. 12 : i-xxvi, 1-554. 1885.
44. MCINTOSH, W. C. Monograph of the British Annelids. Vol. 2, pt. 1. Ray Society, London. 1908.
45. MCINTOSH, W. C. Monograph of the British Annelids. Vol. 3, pt. 1. Ray Society, London. 1915.
46. MCINTOSH, W. C. Monograph of the British Annelids. Vol. 4, pt. 1. Ray Society, London. 1922.
47. MCINTOSH, W. C. Monograph of the British Annelids. Vol. 4, pt. 2. Ray Society, London. 1923.
48. MALMGREN, A. J. Öfv. Kgl. Svenska Vetenskapsakad. Förhandl. No. 5 : 355-410. 1865.
49. MALMGREN, A. J. Öfv. Kgl. Svenska Vetenskapsakad. Förhandl. No. 4 : 127-235. 1867.
50. MARENZELLER, E. Denschr. Kais. Akad. wiss. Wien, Math.-naturw. Klasse, 41(2) : 109–154. 1879.
51. MARENZELLER, E. Ann. K. K. Naturhist. Hofmuseums, Wien, 5 : 1-8. 1890.
52. MONRO, C. C. A. Saertyk Vidensk. Medd. Dansk naturhist. Foren. 85 : 75-103. 1928.
53. MONRO, C. C. A. "Discovery" Repts. 2 : 1-222. 1930.
54. MONRO, C. C. A. Proc. Zool. Soc. London, 1933 : 1-96 and 1039-1092. 1933.
55. MONRO, C. C. A. "Discovery" Repts. 12 : 59-198. 1936.
56. MOORE, J. P. Proc. Acad. Natural Sci. Phila. 55 : 401-490. 1903.
57. MOORE, J. P. Proc. Acad. Natural Sci. Phila. 56 : 484-502. 1904.
58. MOORE, J. P. Proc. Acad. Natural Sci. Phila. 57 : 525-569 and 846-860. 1905.
59. MOORE, J. P. Proc. Acad. Natural Sci. Phila. 58 : 217-259. 1906.
60. MOORE, J. P. Proc. Acad. Natural Sci. Phila. 60 : 321-364. 1908.
61. MOORE, J. P. Proc. Acad. Natural Sci. Phila. 61 : 235-295 and 321-351. 1909.
62. MOORE, J. P. Proc. Acad. Natural Sci. Phila. 62 : 328-402. 1910.
63. MOORE, J. P. Proc. Acad. Natural Sci. Phila. 63 : 234-318. 1911.
64. MOORE, J. P. Proc. Acad. Natural Sci. Phila. 75 : 179-259. 1923.
65. OKUDA, S. Science Repts. Tôhoku Imp. Univ. (Fourth Ser.) 12(1) : 45-69. 1937.
66. PIXELL, H. Proc. Zool. Soc. London, 1912 : 784-805. 1912.
67. POTTS, P. A. Proc. Zool. Soc. London, 1914 : 955-994. 1914.
68. SOUTHERN, R. Proc. Roy. Irish Acad. 31. 1914.
69. STIMPSON, W. Smithsonian Inst. Pub. Contrib. to Knowledge. 1853.
70. TREADWELL, A. L. Bull U.S. Fish Commission, 23(3) : 1147-1181. 1906.
71. TREADWELL, A. L. Univ. Calif. Pub. Zool. 13(8) : 175-234. 1914.
72. TREADWELL, A. L. Carnegie Inst. Wash. Pub. 312 : 171-181. 1922.
73. TREADWELL, A. L. Proc. U.S. Natl. Museum, 67(29) : 1-3. 1925.
74. TREADWELL, A. L. Am. Museum Novitates, No. 223 : 1-8. 1926.
75. TREADWELL, A. L. Zoologica, 22(2) : 139-160. 1937.
76. WEESE, A. O. Proc. Oklahoma Acad. Sci. 13 : 18-21. 1932.

THE EFFECT OF ASPHYXIATION UNDER VARIOUS TENSIONS OF CARBON DIOXIDE ON THE SWIM BLADDER GASES OF SOME FRESHWATER FISH[1]

By Virginia Safford Black[2]

Abstract

Freshwater physostomous fish, possessing open swim bladders (dace, sucker, chub, bullhead, trout), and physoclistous fish, having closed swim bladders (perch, bass, pumpkin-seed, stickleback), were sealed in bottles of water saturated with air and containing various amounts of dissolved carbon dioxide. After asphyxiation, an analysis of the swim bladder gases showed that the swim bladder had gained some carbon dioxide and lost some oxygen. Fish with closed swim bladders possessing *retia mirabilia* and ovals, vascular networks that facilitate gas exchange, show a markedly greater exchange of gases during the experiments than fish with open swim bladders which have no intensely vascular regions on the swim bladder wall. The oxygen that leaves the swim bladder during asphyxiation is probably of no significant value to the fish for respiration.

Introduction

The main function of the swim bladder of fish is that of adjusting the buoyancy of the fish at various depths and pressures. Further functions attributed to it are respiration (16, 20, 22), hearing (3, 18), sound production (25), pressure sensitivity (2, 4), and co-ordination of movement (26). The present paper deals with the exchange of gases at the swim bladder, a process involved in buoyancy adjustments and in respiration.

The experiments show the response of two types of swim bladder to changes in the gas content of the water. One of these types is the open or physostomous swim bladder. It is connected to the anterior gut by an open duct, the *ductus pneumaticus*. Regulation to hydrostatic pressure may be made by the passage of gas to or from the swim bladder by way of the duct. The second type is the closed or physoclistous swim bladder whose duct is closed or completely atrophied at a very early stage in the life of the fish. All gaseous regulation to increased external pressure in these fish must be made by secreting gas into the bladder through the combined effort of the *rete mirabile* and gas gland of the swim bladder. Gas is absorbed again into the blood at the oval, a section of the swim bladder wall whose vascular supply is made available by nervous control when there is a decrease in hydrostatic pressure (17, 19). Some physostomes also have *retia mirabilia*, but none of the physostomous species represented in this paper are in that group.

The proportion of carbon dioxide, oxygen, and nitrogen in the swim bladders of fish living in water equilibrated with air appears to be charac-

[1] *Manuscript received in original form December 1, 1941, and as revised, April 24, 1942. Contribution from the Ontario Fisheries Research Laboratory, Algonquin Park, Ont., and Swarthmore College, Swarthmore, Pa. The research reported in this paper was an integral part of a program being carried out at the Ontario Fisheries Research Laboratory, Algonquin Park, which was supported in part by a grant provided by the National Research Council of Canada.*

[2] *Assistant, Department of Zoology, University of Toronto, Toronto, Ont.*

TABLE I

GAS CONTENT OF THE SWIM BLADDERS OF CONTROL FISH

	Carbon dioxide			Oxygen		
	Average %	σM	Number of fish	Average %	σM	Number of fish
Physoclists:						
Bass	1.4	±0.50	5	26.0	±0.64	5
Pumpkin-seed	0.9	±0.17	10	21.2	±1.2	10
Stickleback	1.2	±0.30	5	19.6	±0.87	5
Perch	1.3	±0.27	12	19.4	±0.71	12
Physostomes:						
Dace	4.9	±0.71	9	12.1	±1.4	9
Sucker	1.9	±0.26	5	11.1	±0.49	5
Bullhead	0.6	±0.08	12	7.5	±0.64	12
Chub	2.4	±0.55	10	6.4	±1.2	10
Trout	0.8	±0.17	13	6.0	±0.71	13

TABLE II

A. COMPARATIVE DATA ON CARBON DIOXIDE AND OXYGEN PERCENTAGES FOUND IN THE SWIM BLADDERS OF CONTROL FISH LIVING IN WATER EQUILIBRATED WITH AIR

Species	No. of fish	CO_2, %	σM	O_2, %	σM	Place of collection	Reference
Perca flavescens	28	0.63	±0.03	19.9	±0.88	Wisconsin	(8)
	12	1.3	±0.27	19.4	±0.71	Ontario	Table I
Micropterus dolomieu	3	2.5	±0.84	24.6	±5.8	Wisconsin	(8)
	5	1.4	±0.50	26.0	±0.64	Ontario	Table I
Lepomis gibbosus	9	0.65	±0.07	21.9	±0.61	Wisconsin	(8)
	10	0.9	±0.17	21.2	±1.2	Ontario	Table I
Catostomus commersonnii	1	4.3	—	5.4	—	Wisconsin	(8)
	21	4.6	±0.36	13.6	±0.27	Pennsylvania	(24)
	5	1.9	±0.26	11.1	±0.49	Ontario	Table I

B. COMPARATIVE DATA FOR PHYSOCLISTS AND PHYSOSTOMES SHOWING A SIGNIFICANT DIFFERENCE IN CARBON DIOXIDE AND OXYGEN PERCENTAGES

	No. of species	CO_2, %	σM	O_2, %	σM		Reference
Physoclists	20	1.0	±0.11	22.2	±2.4		(8, 12, 23) and Table I
Physostomes	15	2.8	±0.45	10.4	±2.6		(8, 12, 24) and Table I
Δ		1.8	±0.46	11.8	±3.5		

teristic of the species (Table IIA), but subject to variation during gas secretion and resorption in response to changes in hydrostatic pressure (8, 11). Since the swim bladders of the physostomes are exposed chiefly to gases in the gut one finds in most species a higher carbon dioxide and lower oxygen percentage (Table IIB) than is present in the water. The physoclists, as shown in Tables I and IIB, usually have a fairly low carbon dioxide percentage and an oxygen percentage close to or higher than that of water saturated with air (21% oxygen). The relation between the gases of the water and swim bladder is probably more marked in the physoclists because gas exchange takes place by way of the blood both at the gills and at the *rete mirabile* and oval of the swim bladder.

It has been shown by experiment that (*a*) when the carbon dioxide in the water is increased the carbon dioxide content of the swim bladder also rises (8, 21), (*b*) when the oxygen in the water is decreased the percentage of oxygen in the swim bladder also decreases (8). In the present paper (*a*) and (*b*) are combined since the carbon dioxide in the water is increased to various tensions and the fish lowers the oxygen tension to the asphyxial point in each.

The asphyxial oxygen tension at the gills is influenced by the tension of carbon dioxide in the water so that when the carbon dioxide is increased beyond a certain tension, characteristic for each species, the oxygen tension at asphyxiation is also higher (6, 7). The changes in the swim bladder gases during asphyxiation in this manner have been followed in some physoclistous marine fish with the following results (23): (*a*) carbon dioxide enters the swim bladder reaching a pressure comparable to the carbon dioxide tension of the water until the latter is so high that it can be tolerated by the fish only a short time, thus decreasing the time for equilibration before death, (*b*) oxygen disappears from the swim bladder during asphyxiation and in a few cases was entirely lacking at the end of the experiment, (*c*) the extent of disappearance of oxygen appeared to depend on the carbon dioxide and oxygen tension of the water, reflecting the condition at the gills.

Experiments carried out in the summer of 1940 on some of the freshwater fish in Algonquin Park, Ont., are reported in this paper and the results compared with those of similar work on marine fish (23).

Materials and Methods

The four species of physoclists used were the northern smallmouth bass, *Micropterus dolomieu* Lacépède; the pumpkin-seed, *Lepomis gibbosus* (Linnaeus); the yellow perch, *Perca flavescens* (Mitchill); the brook stickleback, *Eucalia inconstans* (Kirtland). The physostomes are represented by the finescale dace, *Pfrille neogaea* (Cope); the common white sucker, *Catostomus commersonnii* (Lacépède); the northern creek chub, *Semotilus atromaculatus* (Mitchill); the northern brown bullhead, *Ameiurus nebulosus* (LeSueur); the common brook trout, *Salvelinus fontinalis* (Mitchill). The nomenclature is taken from Hubbs and Lagler (10).

All the species except the sucker and trout were hooked, seined, or trapped in the surface waters of Lake Opeongo or small neighbouring lakes. The suckers were trapped at a depth of about 30 ft. The trout had been raised by the Department of Game and Fisheries of Ontario.

From the point of view of their resistance to asphyxiation the previous history of the fish apparently made little difference, provided that experiments were done at very nearly the temperature from which the fish had come. The swim bladder gases, on the other hand, are influenced by the hydrostatic pressure on the fish. Hence it was desirable to keep the fish caught in deep water (some bass and perch, all suckers) in surface water for a day before using them for experiment. Complete adjustment to pressure could be made in this time (1), so that no gas exchange resulting from change in external pressure would occur during the experiments. Previous to the experiments all the fish were living in lake water containing at all times less than 10 mm. carbon dioxide and over 120 mm. oxygen.

The procedure and analytical methods were identical with those used for marine fish (23), i.e. each fish was asphyxiated in a sealed bottle of water containing carbon dioxide at tensions from 4 to 300 mm. and oxygen at tensions from 100 to 250 mm. depending on the individual experiment (7). After asphyxiation the oxygen in the water was determined by the Winkler method, the carbon dioxide by equilibration of the water with a small bubble of air which was then analysed for carbon dioxide by a modification of Krogh's micromethod (14). The latter method was also used for determining the percentage of carbon dioxide and oxygen in the swim bladder gas. The apparatus used in this work was accurate to 0.5 of 1%. The temperature of the experiments ranged from 17° to 22° C. No effect of temperature was apparent within this range. The swim bladder gases of control fish, caught and kept in the same manner as the fish used for the experiments, were also analysed.

The duration of the experiments varied from one to four hours for all species except two. These were the dace, which sometimes continued respiratory movements as long as five hours, and the bullhead which lived up to seven hours in a sealed bottle. In general the experiments at low carbon dioxide tensions lasted longer than those at high carbon dioxide tensions presumably because the ability of the fish to use oxygen decreased as the carbon dioxide in the water was increased.

The pressures of the swim bladder gases, recorded for comparison with water tensions, have been calculated for Table III and Fig. 1 on the assumption that the gases in the swim bladder were at atmospheric pressure. This was not so in the cyprinoid fishes (chub and dace) nor in the sucker where the pressure within the swim bladder was often sufficient to displace the plunger of the syringe (cf. Reference 3). However, all pressures have been calculated from percentages as follows: the percentage of gas (10% carbon dioxide, for example) was multiplied by the barometric pressure (in mm. mercury) at 1300 ft. less the water vapour pressure (in mm. mercury) at the temperature

of the experiment (725 − 14.5 at 17.0° C.), giving 71.0 mm. carbon dioxide. The laboratory is located at an altitude of 1300 ft.

The curves in Fig. 1 were drawn by inspection of the plotted data. The standard deviation of the mean (σM) is recorded for the data presented in Tables I, II, and IV.

TABLE III

THE RANGE OF PRESSURES OF CARBON DIOXIDE AND OXYGEN FOUND IN THE WATER AND SWIM BLADDER AFTER ASPHYXIATION. A SUMMARY OF ALL THE EXPERIMENTS

	No. of fish	Gases in water		Gases in swim bladder			
		CO_2, mm.	O_2, mm.	CO_2		O_2	
				mm.*	%	mm.*	%
Physoclists:							
Perch	25	5– 88	5–109	7– 75	0.9–10.7	24–131	3.4–18.6
Bass	17	6– 95	12–186	7– 78	0.8–11.1	40–195	3.8–28.4
Pumpkin-seed	20	4–119	5–180	11– 66	1.6– 9.4	23–121	3.4–17.1
Stickleback	20	4–195	13–175	17–202	2.4–28.6	18– 65	2.6– 9.2
Physostomes:							
Sucker	16	7– 99	6–168	14– 37	2.0– 5.2	48– 99	6.8–14.1
Trout	29	5–137	16–203	3– 97	0.4–13.9	3–112	0.4–16.0
Dace	25	6–200	4–172	37–125	5.2–17.8	6–169	0.8–24.0
Chub	25	4–202	5–112	21– 78	3.0–11.1	20–151	2.9–21.6
Bullhead	20	7–300	1–119	5– 82	0.7–11.8	13– 92	1.8–13.1

* *Pressures of swim bladder gases calculated by assuming atmospheric pressure in the bladder.*

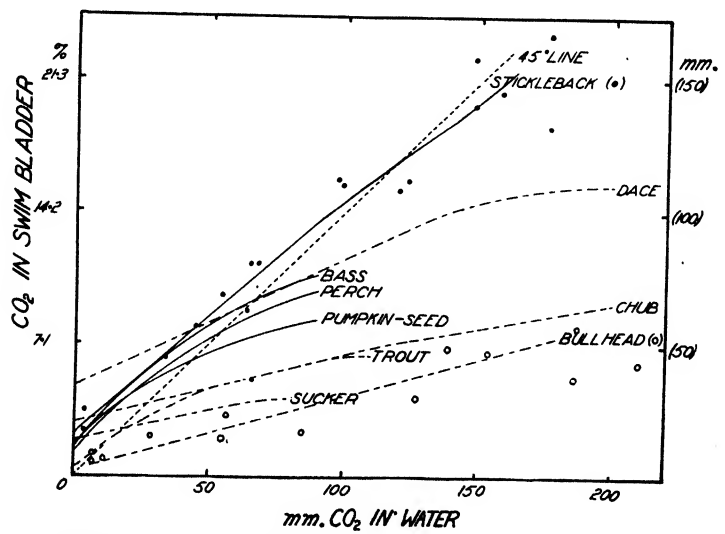


FIG. 1. *Relation of carbon dioxide in water to carbon dioxide in swim bladder after asphyxiation.* ——— = *physoclists;* – – – – = *physostomes.*

Results

The specific nature of the proportion of gases in the swim bladder is brought out in Table II. Here the carbon dioxide and oxygen percentages for control fish (Table I) are compared with the percentages found by Hall (8) for the same species.

The data for all the experiments have been summarized in Table III to show the range of carbon dioxide tensions used for each species, the resulting asphyxial oxygen tensions, and the range of carbon dioxide and oxygen pressures in the swim bladder, all determined after the asphyxiation of the fish. The number of fish used, i.e., the number of single experiments, is indicated. During the experiments the carbon dioxide in the water decreased slightly; the carbon dioxide tension after asphyxiation was rarely less than 4/5 of the initial tension. The oxygen in the water decreased to the asphyxial oxygen level which varied with each species and carbon dioxide tension. The carbon dioxide in the swim bladder increased, the oxygen appeared to decrease or show little change. The table serves chiefly to give a general comparison of the gases in the water with those in the swim bladder and to show the tensions of carbon dioxide that each species can tolerate. The physoclists and physostomes are listed in order of increasing ability to tolerate carbon dioxide.

The relation between the carbon dioxide in the water and in the swim bladder at the time of asphyxiation is given in Fig. 1. The data are plotted for the stickleback and bullhead up to 210 mm. carbon dioxide. The remaining points are beyond the scope of this figure but are included in the tables. It is clear that carbon dioxide enters the swim bladder in all these species but it is also apparent that the physoclists are in closer equilibrium with the water than are the physostomes. The latter show a slow reaction to the carbon dioxide in the water and rarely attain a carbon dioxide pressure in the swim bladder equivalent to that of the water unless it was present initially. Though the line for the dace seems high because of the high initial carbon dioxide in the bladder the actual slope is more gradual than the slope of the physoclists. The marine and freshwater physoclists, however, keep in the neighbourhood of the 45° line until the carbon dioxide in the water becomes so great that asphyxiation occurs before the equilibration takes place. It may be concluded, therefore, that carbon dioxide enters the swim bladder of physoclists more readily than the swim bladder of these physostomes.

The ability of freshwater and marine fish to use the oxygen in the swim bladder during asphyxiation is compared in Table IV. The results of experiments at all carbon dioxide tensions are averaged for each species (Asphyxiated). These averages are compared with the controls (Control), and the actual (ΔO_2) and percentage differences in oxygen are noted. After asphyxiation there is a significant decrease in the oxygen in the swim bladder of all the physoclists except the scup. The physostomes, on the other hand, show no significant difference in the proportion of oxygen in the swim bladder

TABLE IV

CHANGE IN THE OXYGEN CONTENT OF THE SWIM BLADDER IN ASPHYXIATION. AN AVERAGE OF ALL EXPERIMENTS COMPARED WITH THE CONTROLS

	Control			Asphyxiated			ΔO_2	Decrease in O_2, %
	O_2, %	σM	No. fish	O_2, %	σM	No. fish		
Marine fish								
Physoclists:								
Scup	9.4	±1.6	4	7.0	±0.94	8	− 2.4±1.8	25
Cunner	25.8	±1.5	4	13.2	±1.7	19	− 12.6±2.2	49
Killifish	15.6	±2.3	16	7.3	±0.51	22	− 8.3±2.4	53
Tautog	51.3	±3.4	4	14.6	±4.6	11	− 36.7±5.7	72
Toadfish	55.0	±3.2	32	15.0	±4.2	16	− 40.0±5.1	73
Sea robin	22.8	±1.7	6	3.6	±0.91	8	− 19.2±1.9	84
Freshwater fish								
Physoclists:								
Bass	26.0	±0.64	5	17.6	±1.4	17	− 8.4±1.4	32
Perch	19.4	±0.71	12	10.7	±0.88	25	− 8.7±1.1	45
Pumpkin-seed	21.2	±1.2	10	9.7	±1.1	20	−11.5±1.6	54
Stickleback	19.6	±0.87	5	5.6	±0.54	20	−14.0±1.0	72
Physostomes:								
Chub	6.4	±1.2	10	8.9	±0.95	25	+ 2.5±1.6	—
Trout	6.0	±0.71	13	6.4	±0.71	29	+ 0.4±1.0	—
Sucker	11.1	±0.49	5	9.7	±0.53	16	− 1.4±0.72	13
Dace	12.1	±1.4	9	9.7	±1.3	25	− 2.4±2.0	20
Bullhead	7.5	±0.64	12	6.0	±0.58	20	− 1.5±0.87	20

of control and asphyxiated fish, and in no case is the percentage decrease in oxygen as high as the lowest value found in the physoclists. It appears that little, if any, oxygen in the swim bladder is available to the physostomes whereas the physoclists lose from 25 to 84% of their swim bladder oxygen during asphyxiation.

The above paragraphs present the general effect of asphyxiation on the swim bladder gases when carbon dioxide is present in the water. The course of the individual experiments for the physostomes, with respect to carbon dioxide, is shown in Fig. 1. No trend is consistently exhibited in the swim

Carbon dioxide in water	Carbon dioxide in swim bladder		Oxygen in swim bladder	
	Freshwater fish	Marine fish	Freshwater fish	Marine fish
None added	Low	Low	High	Low
Intermediate	Increasing	Increasing	Low	Increasing
High	High or variable	High or variable	High (i.e. near normal O_2 percentage)	High or variable

bladder oxygen with an increase in the carbon dioxide in the water or swim bladder. The gases in the physoclist swim bladder, however, assume the following pattern as the carbon dioxide in the water is increased.

Hence it is apparent that the carbon dioxide in the swim bladder is directly controlled by the carbon dioxide in the water, but it is difficult to see what factor is responsible for the changes in oxygen. The difference between the proportion of oxygen in the swim bladder of freshwater and marine fish at low carbon dioxide tensions is significant, and shows that a factor other than simple diffusion determines the oxygen in the swim bladder. Since freshwater fish have a higher alkaline reserve than marine fish (5), small amounts of carbon dioxide would have less effect on the internal environment; the situation for freshwater fish at intermediate carbon dioxide tensions might therefore actually be equivalent to that of marine fish at low carbon dioxide tensions.

The stickleback (average weight, 0.6 gm.; volume of gas, about 0.05 cc.) differs from the other freshwater physoclists in the consistently low oxygen content of its swim bladder after asphyxiation no matter how high the carbon dioxide or oxygen in the water. This high utilization of swim bladder oxygen in asphyxiation may be due to respiration of the cells of the swim bladder wall or neighbouring tissues. Changes brought about in this way might show up in the stickleback because of the very small volume of gas. However, a similar situation occurs in the swim bladder of the marine fish, the sea robin, which is a good-sized fish with an exceptionally well developed *rete mirabile* (average weight of sea robin, 120 gm.; volume of gas, 10 to 15 cc.).

Discussion

The data show that during asphyxiation a change occurs in the proportion of gases in the swim bladder toward equilibration with the gas content of the water. The change is more marked in the physoclistous fish than in the physostomous species. Two questions arise from these facts: (*a*) the method of passage of gases to and from the swim bladder, (*b*) the reason for the difference in the quantity of exchange in the two types.

Gas can enter or leave the swim bladder either by passing through the swim bladder wall or by diffusing to and from the blood. (Passage of gas through the duct of the physostomes was not a factor in the experiments.) There are conflicting ideas about the permeability of the swim bladder wall to gases and it is possible that permeability varies with the species for there is great morphological variation. In any case, even in the swim bladders that appear permeable, carbon dioxide is the gas that most readily passes the wall, oxygen taking weeks to diffuse out (11, 15). The data for the physostomes indicate little change in the percentages of the gases during asphyxiation. Therefore loss or gain through the swim bladder wall cannot be large during the time of the experiments. Since the data for the physoclists show a very appreciable decrease in oxygen within one to four hours, the greater proportion of gas exchange must be by way of the blood.

The physostome swim bladder is supplied with blood vessels that are distributed over the swim bladder wall so that it is by no means lacking in blood supply. However, these species do not possess the specialized vascular networks found in the physoclists. Precisely how and when the networks function in the course of these experiments is a matter of conjecture. Normally the oval absorbs gas from the swim bladder and it is probable that carbon dioxide enters at both the oval and *rete mirabile* in the experiments.

Other factors that undoubtedly affect the gas exchange at high carbon dioxide tensions are: (*a*) higher oxygen tension in the water at asphyxiation, (*b*) less time for equilibration because of shorter duration of the experiments, (*c*) the physiological effect of carbon dioxide in the swim bladder on the combination and dissociation of oxygen and blood, (*d*) decrease in heart-beat rate (9), resulting in slower circulation.

It is apparent from Table IV that the average percentage of oxygen in the swim bladder of asphyxiated fish is similar for both physoclists and physostomes. It might be argued that the physoclists absorb a higher percentage of oxygen only because they have more to begin with, whereas the physostomes reach the same "minimum" by absorbing very little oxygen. The following considerations indicate an *actual* greater absorption of oxygen by physoclists than by physostomes. (*a*) The experimental physostomes show as much variation as the controls, but the oxygen in the swim bladders of the physoclistous fish is influenced by the experimental conditions. (*b*) The fact that carbon dioxide does not readily enter the physostome swim bladder might indicate that oxygen does not readily disappear. (*c*) Khalil (13) found that a carp (physostome) whose swim bladder oxygen had been artificially enriched to 26.3% absorbed 3% in five hours when in oxygen-poor water. The oxygen content of the control bass averages 26% (Table IV), the asphyxiated bass averaging 17.6% in experiments lasting less than five hours. In a comparable situation then the physostome absorbed only 3%, whereas the physoclist absorbed 8% of its swim bladder oxygen.

The disappearance of some of the oxygen from the swim bladder of the physoclists brings up the question of the use of the swim bladder as a source of oxygen in respiratory stress. The function of the swim bladder as an accessory organ of respiration has been clearly shown by Potter (20) for some of the bony ganoid fishes with open swim bladders, such as the gar-pike and bowfin, which come to the surface and gulp air into the bladder. The structure of their swim bladders is more complex and the circulation adapted for respiration. The teleost physostome, *Umbra krameri*, a European freshwater fish, also uses its swim bladder as an accessory organ of respiration but its swim bladder is provided with gas gland, *rete mirabile*, and vascular epithelium (22). The swim bladders of the teleost physostomes of this paper, however, do not show any adaptation for a respiratory function. In the physoclists the facility of gas exchange by means of the blood is a necessity for regulation to external pressure since there is no other way for gas to enter or depart. It seems plausible, therefore, that oxygenated blood leaving the

swim bladder might serve to benefit other tissues. However, the availability of the oxygen to other tissues for respiration appears unlikely since the blood leaving the swim bladder goes either directly, or by way of the liver, to the heart and thence to the gills for oxygenation. En route it is joined by vessels of deoxygenated blood from the rest of the body so that its effectiveness as an oxygen carrier is shortlived and inefficient. Experiments and calculations by Hall (8) show that only about 1 cc. of oxygen disappears from the swim bladder of perch kept in water of low oxygen content for five hours, the oxygen consumption of the fish being about 6 cc. per hr. Likewise extensive work by Khalil (13) on the significance of the swim bladder in respiration indicates that the Europen tench and carp (physostomes) kept in oxygen-poor water for seven or eight hours, absorb from the swim bladder only the amount of oxygen that would be equivalent to the oxygen consumption of the fish over a period of three or four minutes.

The experiments reported in this paper show that the gases in the physostome swim bladder are fairly independent of the gases in the water and that the oxygen in the swim bladder is not readily mobilized during asphyxiation. The gases of the physoclist swim bladder, however, are in greater harmony with the external and probably the internal environments. Oxygen decreased during asphyxiation though its value to these fish for respiration is probably insignificant.

Acknowledgments

The author is greatly indebted to Dr. F. E. J. Fry and to Dr. E. C. Black for their assistance and co-operation.

References

1. Black, V. S. Unpublished data. 1940.
2. Evans, H. M. Proc. Roy. Soc. (London) B, 97 : 545-576. 1925.
3. Evans, H. M. and Damant, G. C. C. Brit. J. Exptl. Biol. 6 (1) : 42-55. 1928.
4. Fiebiger, J. Verhandl. Zool.-bot. Ges. 73 : 141-147. 1924.
5. Fontaine, M. and Boucher-Firly, S. Bull. inst. océanograph. (Monaco). No. 646 : 1-12. 1934.
6. Fry, F. E. J. Am. Assoc. Advancement Sci. Pub. No. 10 : 132-142. 1939.
7. Fry, F. E. J. and Black, E. C. Anat. Record, 72 (Suppl.) : 47. 1938.
8. Hall, F. G. Biol. Bull. 47 : 79-118. 1924.
9. Haywood, C., Stevens, T. O., Tewinkel, H. M., and Schott, M. J. Cellular Comp. Physiol. 5 (4) : 509-518. 1935.
10. Hubbs, C. L. and Lagler, K. F. Cranbrook Inst. Sci. Bull. 18. 1941.
11. Jacobs, W. Z. vergleich. Physiol. 18 : 125-156. 1932.
12. Jacobs, W. Z. vergleich. Physiol. 20 : 674-698. 1934.
13. Khalil, F. Z. vergleich. Physiol. 25 : 256-282. 1937.
14. Krogh, A. Skand. Arch. Physiol. 20 : 279-288. 1908.
15. Ledebur, J. v. Biol. Rev. Cambridge Phil. Soc. 12 (2) : 217-244. 1937.
16. Leiner, M. Die physiologie der fischatmung. Akademische Verlagsgesellschaft, Leipzig. 1938.
17. Meesters, A. and Nagel, F. G. P. Z. vergleich. Physiol. 21 : 646-657. 1934.
18. Nusbaum, J. Zool. Anz. 4 : 552-556. 1881.

19. NUSBAUM, J. Anat. Anz. 31 : 169-174. 1907.
20. POTTER, G. E. J. Exptl. Zool. 49 (1) : 45-67. 1927.
21. POWERS, E. B. *et al.* Ecol. Monographs, 2 : 385-473. 1932.
22. RAUTHER, M. Zool. Jahrb. Abt. III, 34 : 339-364. 1914.
23. SAFFORD, V. J. Cellular Comp. Physiol. 16 (2) : 165-173. 1940.
24. SAFFORD, V. Unpublished data. 1939, 1940.
25. TOWER, R. W. Ann. New York Acad. Sci. 18 : 149-180. 1908.
26. WASSILENKO, F. D. J. Physiol. U.S.S.R. 23 : 310-313. 1937.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. AUGUST, 1942 NUMBER 8

POLARIZATION AND PROGRESSION IN PAIRING

II. PREMEIOTIC ORIENTATION AND THE INITIATION OF PAIRING[1]

BY STANLEY G. SMITH[2]

Abstract

Homologous chromosomes in the Diptera associate side by side in pairs at each and every anaphase (somatic pairing) and reappear in the following prophases relationally coiled. In plants and animals other than Diptera the homologues at anaphase (with one exception) show no such specific attraction: at prophase the relational coiling of homologues is here supplanted by a relational coiling of sister chromatids. The one exception arises at the anaphase of the last premeiotic division—homologues become associated in pairs and reappear in the following prophase relationally coiled.

In the Diptera the chromosomes are single at each and every anaphase: in other animals and plants the chromosomes are double at all anaphases except that of the last premeiotic division. Hence at this latter division the attraction in pairs between chromatids is replaced by an attraction between pairs of homologues.

That somatic pairing is characteristic of the Diptera has been known since the pioneer work of Stevens (67) and Metz (39). Stevens stated with regard to nine species that "In many cases the prophases of spermatogonia and first spermatocytes resemble each other very closely, the members of each pair being twisted together in both.", and pointed out that "In the spermatocyte we get complete synapsis and reduction; in the spermatogonium only a foreshadowing of reduction . . .". Metz found association of homologues at metaphase and anaphase of diploid mitoses in about 80 species, from the lowest to the highest of the order. Metz and Nonidez (41) working on *Asilus sericeus* noted that in spermatogonial prophase ". . . each aggregate gives rise to a long double thread by a process of attenuation or uncoiling (fig. 1)." At the final spermatogonial anaphase the chromosomes go to the poles associated in pairs, and the late anaphase crowding makes pairing more intimate. The crowding is relaxed at telophase and the pairs are seen to be intimately associated, so much so, ". . . that the duality is often obscured." The resting stage is very brief, no leptotene was observed, and no doubleness was visible, except as paired chromosomes. In *Asilus notatus* similar conditions were found to hold, and the authors were led to state ". . . it may be possible to bring all types [of Diptera] within a common general scheme in which synapsis occurs in the anaphase or telophase of the final spermatogonial division." (42).

[1] *Manuscript received May 23, 1942.*

Contribution No. 2183, Division of Entomology, Science Service, Dominion Department of Agriculture, Ottawa, Canada.

[2] *Presently stationed at, and Research Associate in, McGill University, Montreal, Que.*

A study now in progress of two dipterous parasites, *Bessa selecta* and *Phorocera hamata* (?), has shown these same general features in somatic mitoses. The pairs of homologous chromosomes are associated closely at mitotic prophase by relational coiling (*contra* Darlington (12, p. 235)); as far as can be seen the individual chromosomes comprising each pair are single until late prophase when they become uncoiled, visibly double, and associated only at the centromeres. Visible division of the centromeres is followed by separation of the chromatids of the paired chromosomes, those of one moving to opposite poles each closely paralleled by a chromatid from the other chromosome. By telophase these pairs are intimately associated side by side and reappear in the next prophase again relationally coiled.

Within the last two years four papers have appeared which indicate that the chromosomes in some liliaceous plants are polarized at the prophase of meiosis (6, 13, 21, and 65) as has been demonstrated by Belar (7) in *Tradescantia*. Direct demonstration of polarization is difficult in most Liliaceae, presumably on account of the great length of the chromosomes at early prophase. Darlington (12, p. 91) originally denied the occurrence of polarization in ". . . some Liliaceae except merely as a relic of the orientation of the chromosomes, with their centromeres towards the pole, at the preceding telophase . . ." Apparently he considered polarization to be an active process of orientation during meiotic prophase, and, moreover, still does so (13). The occurrence of this "active" polarization is questionable–Gelei (23, 24) to the contrary; in any case, the evidence of immobility during the resting stage points to a polarization resulting from the last premeiotic anaphase separation (designated by Gelei (23, 24), Rabl's (58) orientation); the centromeres are grouped together against the nuclear membrane and the distal ends are concomitantly grouped at the opposite side of the cell (65). Such an orderly arrangement appears to be a prerequisite to orderly pairing. It is presumed that following spatial derangement the bivalents become interlocked (13, 65). Interlocking, however, is tolerably rare, certainly rarer than could possibly be expected unless the position of homologues were predetermined, or unless pairing were initiated at one point only, the centromere, and progressed outwards from that point. Therefore the question is, are the positions assumed by pairing homologues predetermined? A consideration of the literature supplies a reasonable answer (Table I).

As a result of work on dipterous parasites, on sawflies, grasshoppers, and various plants, the writer has been led to the view that in the premeiotic telophase there is an active mutual selection of maternal and paternal homologues during their movement towards the poles. This concept recalls to mind the statement of Wilson (76) regarding somatic pairing:— "It seems quite possible that the way for synapsis may be prepared already in a very early pre-synaptic stage, by a definite regrouping of the chromosomes that may take place before the leptotene loops are formed as such." Supporting evidence for this thesis comes largely from the literature, although it has been pointed out earlier (64) that there is no characteristic leptotene at oogenesis

TABLE I

OCCURRENCE OF PREMEIOTIC ASSOCIATION OF HOMOLOGOUS CHROMOSOMES

(i) Prophase of second cleavage	Arthropoda, Insecta (Diptera)	*Drosophila melanogaster*	Huettner (28)
(ii) All embryonic nuclei	Arthropoda, Insecta (Diptera)	*Drosophila "ampelophila"*	Stevens (67)
		Many species	Metz (39)
(iii) Probably all somatic nuclei	Arthropoda, Insecta (Diptera)	*Culex pipiens*	Taylor (73); Hance (26)
		Ca. 80 species	Metz (39)
(iv) All gonial metaphases and anaphases	Arthropoda, Insecta (Hymenoptera)	*Apis mellifica*	Nachtsheim (51)
	(Diptera)	Various species	Stevens (67, 69); Metz (39)
		Culex pipiens	Whiting (75)
	Rotifera	*Asplanchna intermedia*	Tauson (72)
(v) Several gonial metaphases	Annelida, Polychaeta (Errantia)	*Ophryotrocha puerilis*	Dehorne (14)
	Arthropoda, Insecta (Orthoptera)	*Syrbula*	Montgomery (47)
		Various species	Eisentraut (18)
	(Hemiptera)	Various species	Montgomery (48)
	Vertebrata, Amphibia (Anura)	*Alytes obstetricus*	Janssens and Willems (32)
	(Urodela)	*Plethedon*	Montgomery (46)
		Salamandra	Dehorne (15)
(vi) Last (?) gonial metaphase	Arthropoda, Insecta (Hemiptera)	Three genera	Slack (63)
	(Hymenoptera)	*Pteronidea ribesii*	Peacock and Sanderson (56); Sanderson (60)
	Mollusca, Gasteropoda (Pulmonata)	*Helix pomatia*	Ancel (1)
	Vertebrata, Marsupiala	Five genera	Koller (33)
(vii) Last gonial anaphase–telophase	Coelenterata, Cnidaria (Hydrida)	*Hydra*	Downing (16)
	Platyhelminthes, Turbellaria	*Dendrocoelum lacteum*	Gelei (22); (retracted (23))
	Annelida, Chaetopoda (Oligochaeta)	*Allolobophora foetida*	Foot and Strobell (20)
	Arthropoda, Onychophora	*Peripatus balfouri*	Montgomery (44)
	Crustacea	Various species	Nichols (53, 54)
	Myriapoda (Chilopoda)	*Scolopendra*	Blackman (9)
	Insecta (Orthoptera)	Various species	McClung (35, 36)
		Dissosteira carolina	Nebel and Ruttle (52)
		Brachystola magna	Sutton (71)
	(Dermaptera)	*Forficula auricularia*	Callan (10)*
	(Hemiptera)	Various species	Montgomery (45)
	(Coleoptera)	*Blaps lusitanica*	Nonidez (55)
	(Hymenoptera)	Three species	Smith (unpub.)
	(Diptera)	Various species with chiasmata	Stevens (67, 68); Moffett (43)
		Various species without chiasmata	Metz (40); Metz and Nonidez (41, 42)
	Arachnida (Araneida)	*Schizocosa crassipes*	Hard (27)
	Polyzoa, Endoprocta	*Pedicellina americana*	Dublin (17)
	Chaetognatha	*Sagitta bipunctata* ♂	Stevens (66)
(viii) After last gonial division but "before conjugation"	Platyhelminthes, Turbellaria	*Planaria lactea*	Arnold (3)
	Arthropoda, Insecta (Hemiptera)	*Euschistus variolarius*	Foot and Strobell (19)
		Corixa distincta	Prokofiewa (57)
	(Coleoptera)	*Hydrophilus piceus*	Arnold (2)
		Tenebrio molitor	Stocking (70)
	(Hymenoptera)	*Diprion polytomum*	Smith (64)
	Chaetognatha	*Sagitta bipunctata* ♀	Stevens (66)

* *Only the sex chromosomes were seen to pair at telophase, at which time they are positively heteropycnotic. It would be of interest to know whether the autosomes associate at the anaphase–telophase of this division.*

in the sawfly, *Diprion polytomum*; a statement later supported by the observation of apparent association of chromosomes in pairs in the last oogonial telophase (unpublished data).

That an association of homologues occurs at or by telophase of the last premeiotic division has been claimed by a number of authors (Table I), while the future assumption of such an association can be inferred from the observation of somatic pairing at metaphase by others. Regarding this, Stevens (66) states "These figures [spermatogonial divisions with the reduced number of chromosomes at telophase, see Fig. 19B] lead me to think that the so-called synapsis stage occurs in *Sagitta* at the close of the final spermatogonia division, the chromosomes uniting in pairs at the poles of the spindle." Of the bouquet stage in oogenesis she states "The regular arrangement of loops in such oocytes as are shown in Figs. 5 and 6, indicate the possibility that they may have begun their development after the last oogonia division without an intervening resting stage, and that the reduction in number to nine bivalent chromosomes may have recently taken place." She notes that she cannot be sure that a resting stage does not intervene between the union in pairs and the bouquet stage, where the nine loops ". . . have a somewhat crenate or beaded outline." (i.e., appear to be relationally coiled). In their later paper, which appears to have been completely overlooked in recent publications, Foot and Strobell (20) have unequivocal evidence for pairing at telophase in *Allolobophora foetida*. They state "Photo 1 shows an oögonial prophase in which 22 chromosomes can be clearly counted and Photo 2 is a young oöcyte in which 11 chromosomes can be counted with equal clearness. We have placed these Photos side by side in order to facilitate a comparison of the two groups of chromosomes. It would seem that such a comparison ought to throw some light on the method of pseudo-reduction, but it is evident this is not the case. The bivalent chromosomes of Photo 2 indicate merely that they have passed through some profound changes between the stages shown in Photos 1 and 2—changes which have presumably occurred after the telophase of the last oögonial division. We have, however, been unable to find any satisfactory evidence of the occurrence of these intervening stages. The strongest evidence we have that the bouquet stage is preceded by a rest stage, is the fact that a nucleolus . . . is frequently found in the bouquet nucleus." The reduced chromosomes are found at the extreme proximal end of the ovary on or near the periphery and ". . . in such close relationship to the oögonial metaphases that the indications are that they arise without the intervention of a typical resting stage." To the writer their Photo 2 represents the telophase of the last oogonial division. The polarized telophase chromosomes ". . . become longer, thinner and *more twisted* [present writer's italics] until finally they disappear and we have the typical resting oöcyte nucleus." All these stages occur in the ovariole. Then there follows a "leptotène, pachytène, and diplotène" in eggs liberated into the receptaculum ovorum ". . . and it is only exceptionally that the largest eggs at the extreme

distal end of the ovary have developed even to the leptotène stage". Hence there can be no doubt regarding the seriation of the stages.

The demonstration of telophase pairing in the Coelenterata, Platyhelminthes, Annelida, Arthropoda, Polyzoa, and Chaetognatha, etc., the occurrence of earlier conditions doubtless leading to it (Table I), and the observation of polarization in the majority of animals and many plants, taken together with deductive evidence for such an orientation in other plants, lead the writer to conclude that pairing in the premeiotic telophase is in some degree or other, the general rule for meiosis. But how could the telophase pairing be conditioned? Further consideration of the Diptera offers a tentative answer which is in agreement with numerous observations.

The homologous chromosomes of the Diptera associate in pairs at anaphase of mitosis whether in somatic, spermatogonial, or oogonial nuclei. The pairs in somatic prophase are relationally coiled and polarized ". . . suggesting the previous anaphase condition" (26). Somatic pairing is established in the egg before cleavage is completed (39), and according to Huettner (28) is visible for the first time at the prophase of the second cleavage division in *Drosophila melanogaster*. There is no attraction between the maternal and paternal homologues on the first cleavage metaphase plate, nor, in the fusion of the polar bodies, do the homologues, seen to be double as they emerge from the resting stage, show any regular attraction for one another, unless the usual arrangement of the three minute fourth chromosomes in the centre of the group be due to attraction. It appears probable (unpublished data) that the attraction between maternal and paternal homologues is initiated during the anaphase of the first cleavage. This, if true, is very significant for the paternal chromosomes at least have been double since the sperm resting stage (50), and have become single only at this anaphase. Hence, one is led to conclude that the late anaphase pairing (and all future somatic pairing) is conditioned by and compensates for the prior anaphase disjunction and consequent singleness of the chromatids.

Darlington and his co-workers consistently deny the doubleness of mitotic anaphase chromosomes (even though it is as consistently seen by numerous competent observers), maintaining that the doubleness arises during the resting stage. Observations to the contrary are ascribed to viewing a cylinder in optical longitudinal section. Huskins and Smith (31), among others, have demonstrated that in *Trillium erectum* the chromonemata are double while jointly helical—thus adequately meeting Darlington's criticism—so that bivalent chromosomes at metaphase are composed of eight threads. The leptotene chromosome is nevertheless single. To reconcile this apparent dualism Huskins (29) suggested that the splitting of the chromonema may be retarded in the last premeiotic division, but found that although this concept led to the resolution of certain difficulties, others then appeared.

Now if the Darlington school were correct in their contention that anaphase chromosomes are single and that attraction is effective only between pairs of single chromosomes, then the homologues should associate in pairs at each

anaphase and telophase[1], as they do in the Diptera (where the writer assumes they are single[2]), or else the pairing observed at pachytene would need be conditioned by some force other than attraction. The fact that somatic pairing does not ordinarily occur in animals other than Diptera, nor in plants, if such forms as *Spinacea* (25) are disregarded, is in agreement with the doubleness of their anaphase chromosomes.

Following Metz (40), Darlington (11) has concluded from an analysis of the conditions in *D. pseudo-obscura* that the association of the homologous autosomes at first meiotic metaphase is due to a specially exaggerated property of attraction in the Diptera. White (74), however, has described an anomalous type of meiosis in a mantid, *Callimantis antillarum*, in which, although chiasmata are absent, the chromosomes nevertheless remain paired. There is in this case no specially exaggerated property of attraction, for there is no somatic pairing either in spermatogonial *metaphases* or those of interstitial cells. White is led to state:— "It seems probable, therefore, that the chromosomes remain associated in bivalents right up to metaphase simply because the split (which normally appears at the end of pachytene) is, in *Callimantis*, delayed until later. Possibly . . . a 'pellicle' holds the chromosomes together."

If the alternative view is accepted, that the chromosomes are invariably double at anaphase, the conditions of singleness at leptotene, observed by such workers as Belar, Belling, and Newton, would demand a healing of the premeiotic split. Duality of the last premeiotic *telophase* chromosomes has been reported in Orthoptera by McClung (36), Robertson (59), and Nebel and Ruttle (52), while duality at the last premeiotic *anaphase* has been figured by Atwood (4) in *Gaillardia aristata* (Compositae). This author illustrates the doubleness at all stages from late metaphase of the last premeiotic division through the resting stage up to zygotene, but, because at no stage are the chromomeres resolved and because at no stage are all the chromosomes countable, the evidence is considered to be unconvincing. McClung's illustrations of Orthoptera chromosomes (and to a lesser extent those of Nebel and Ruttle) appear to establish both duality and telophase pairing, although McClung says of his own material:— ". . . these are not entirely satisfactory or convincing." Of more importance is the fact that no evidence is submitted that the doubleness is present before telophase, i.e., at the time of the establishment of pairing. In any case the whole significance of these observations hinges upon a determination of the positions of the centromeres and the positions of the chiasmata in relation to them (cf. 37).

[1] *That it is not the fact that the anaphase chromosomes are somatically coiled that is responsible for this failure of anaphase pairing follows from Barber's (5) contention that the attraction due to ". . . radial symmetry . . . will not be satisfied by union in twos but, as in the diplochromosomes, may accommodate more."*

[2] *The apparently contradictory evidence of doubleness found by Dr. B. P. Kaufmann ("Somatic Mitoses of Drosophila melanogaster." J. Morphol. 56 : 125-155. 1934.), is here reconciled by the assumption that the "giant cells" are tetraploid. Note the difference in type of "split" and in degree of somatic pairing in the giant and smaller cells of the ganglia.*

Belling (8) states that he does not regard as proved Darlington's conclusion that "Meiosis differs from mitosis in the nucleus entering prophase before the chromosomes divide instead of after they divide." The observation of leptotene doubleness is in agreement with the conditions observed in Orthoptera. Observations of leptotene singleness in other organisms are, the writer believes, not incompatible if the degree of telophase pairing is variable and somewhat inversely correlated with chromosome length (cf. 30). In no case at leptotene should the whole length of the chromosome be normally unpaired; the shorter the chromosome the more complete the pairing. The observation of pairing at preleptotene stages may in this way serve to harmonize some of the conflicting interpretations regarding chromosome structure at leptotene (see especially 20).

These considerations lead to the following conclusions:— (1) the meiotic pairing consummated at pachytene is initiated at the latest by the telophase of the last premeiotic division; (2) this is conditioned by the total (though in some cases only temporary) suppression of the split in the last division; following the disjunction of single chromatids in the last anaphase there is a compensating union of daughter homologous chromatids. Table I makes it clear that the regrouping of chromosomes preparatory to the association of homologues at the last premeiotic telophase may in some organisms (Sections iv, v, and vi) be a gradual process. Presumably the division of the chromosomes *per se* may be incompletely co-ordinated with the division of the cell. When the rate of division of the cell is rapid (as it is in the gonia), that of the chromosomes lags behind and allows partial attraction of homologues; this being necessarily cumulative, somatic pairing in various degrees may become established before the final telophase*.

The most that can be said for these considerations is that they may constitute general rules; exceptions are apparent, for example among coccids, other Hemiptera (61, 62), and Hepaticae (34).

Telophase pairing considered in relation to variability in the time of splitting and assumption of the relational coil has an obvious bearing on such diverse phenomena as "asynapsis", polytene chromosomes, somatic reduction, and diploid parthenogenesis; these will be discussed elsewhere. Here it may be pointed out that the absence of quadrivalents from isolated tetraploid nuclei, e.g. in *Podisma mikado* (38) and in *Forficula auricularia* (10) is not critical evidence that fusion of two diploid nuclei had occurred *after* pachytene (cf. 12, p. 65), but would be the natural consequence of syndiploidy following the establishment of two polarization centres at the anaphase of the last spermatogonial division.

* *In the hermaphroditic coccid, Icerya purchasi, Dr. S. Hughes-Schrader (Z. Zellforsch. mikroskop. Anat. 6 : 509-540. 1927.) has shown that certain cells of the gonad undergo reduction and become haploid during the early divisions of the spermatogonia and that at spermatogenesis only one meiotic division occurs. No evidence on the cytological mechanism underlying this reduction was found, but it is clearly compatible with the scheme outlined here.*

Acknowledgments

It is a pleasure for the writer to state his gratitude to Prof. Franz Schrader and Prof. C. L. Huskins for expressions of opinion regarding the manuscript and to Dr. G. F. Sander for assistance with the literature.

References

1. ANCEL, P. Arch. biol. 19 : 389-652. 1903.
2. ARNOLD, G. Arch. Zellforsch. 2 : 181-190. 1908.
3. ARNOLD, G. Arch. Zellforsch. 3 : 431-448. 1909.
4. ATWOOD, S. La Cellule, 46 : 391-409. 1937.
5. BARBER, H. N. Proc. Roy. Soc. (London) B, 128 : 170-185. 1940.
6. BARBER, H. N. J. Genetics, 42 : 223-257. 1941.
7. BELAR, K. Die cytologischen Grundlagen der Vererbung (Handb. Vererbungswiss. 1). Gebrüder Borntraeger, Berlin. 1928.
8. BELLING, J. Univ. Calif. Pub. Botany, 17(5) : 75-110. 1933.
9. BLACKMAN, M. W. Bull. Museum Comp. Zool. Harvard, 48 : 1-137. 1905.
10. CALLAN, H. G. J. Genetics, 41(2 and 3) : 349-374. 1941.
11. DARLINGTON, C. D. Genetics, 19 : 95-118. 1934.
12. DARLINGTON, C. D. Recent advances in cytology. 2nd ed. J. & A. Churchill, London; P. Blakiston's Son and Company, Philadelphia. 1937.
13. DARLINGTON, C. D. Ann. Botany (n.s.), 5(18) : 203-216. 1941.
14. DEHORNE, A. Zool. Anz. 36 : 209-222. 1910.
15. DEHORNE, A. Arch. Zellforsch. 6 : 613-639. 1911.
16. DOWNING, E. R. Zool. Jahrb. 21 : 379-426. 1905.
17. DUBLIN, L. I. Ann. New York Acad. Sci. 16(1) : 1-64. 1905.
18. EISENTRAUT, M. Z. wiss. Zool. 127 : 141-183. 1926.
19. FOOT, K. and STROBELL, E. C. Biol. Bull. 16 : 215-238. 1909.
20. FOOT, K. and STROBELL, E. C. Arch. Zellforsch. 5 : 149-165. 1910
21. FRANKEL, O. H. J. Genetics, 41(1) : 9-34. 1940.
22. GELEI, J. Arch. Zellforsch. 11 : 51-150. 1913.
23. GELEI, J. Arch. Zellforsch. 16 : 88-169. 1921.
24. GELEI, J. Arch. Zellforsch. 16 : 299-370. 1922.
25. GENTCHEFF, G. and GUSTAFSSON, A. Hereditas, 25 : 349-358. 1939.
26. HANCE, R. T. J. Morphol. 28 : 579-591. 1917.
27. HARD, W. L. J. Morphol. 65 : 121-153. 1939.
28. HUETTNER, A. F. J. Morphol. Physiol. 39 : 249-265. 1924.
29. HUSKINS, C. L. Trans. Roy. Soc. Can. (Ser. 3) 26(Sec. 5) : 17-28. 1932.
30. HUSKINS, C. L. and SMITH, S. G. J. Genetics, 28(3) : 397-406. 1934.
31. HUSKINS, C. L. and SMITH, S. G. Ann. Botany, 49(103) : 119-150. 1935.
32. JANSSENS, F. A. and WILLEMS, J. La Cellule, 25 : 151-178. 1909.
33. KOLLER, P. C. J. Genetics, 32 : 451-472. 1936.
34. LORBEER, G. Jahrb. wiss. Botan. 80 : 567-818. 1934.
35. MCCLUNG, C. E. Kansas Univ. Sci. Bull. 1(8) : 185-231. 1902.
36. MCCLUNG, C. E. J. Morphol. Physiol 43(2) : 181-265. 1927.
37. MCCLUNG, C. E. Z. Zellforsch. mikroskop. Anat. 7(5) : 756-778. 1928.
38. MAKINO, S. Japan. J. Genetics, 15(2) : 80-82. 1939.
39. METZ, C. W. J. Exptl. Zool. 21 : 213-279. 1916.
40. METZ, C. W. Z. Zellforsch. mikroskop. Anat. 4(1) : 1-28. 1926.
41. METZ, C. W. and NONIDEZ, J. F. J. Exptl. Zool. 32 : 165-185. 1921.
42. METZ, C. W. and NONIDEZ, J. F. Arch. Zellforsch. 17 : 438-449. 1923.
43. MOFFETT, A. A. Cytologia, 7(1 and 2) : 184-197. 1936.
44. MONTGOMERY, T. H. Zool. Jahrb. 14 : 277-368. 1900.

45. MONTGOMERY, T. H. Proc. Acad. Natural Sci. Phila. 53 : 261-271. 1901.
46. MONTGOMERY, T. H. Biol. Bull. 6 : 137-158. 1904.
47. MONTGOMERY, T. H. Proc. Acad. Natural Sci. Phila. 62 : 162-205. 1905.
48. MONTGOMERY, T. H. Trans. Am. Phil. Soc. 21 : 97-174. 1906.
49. MOORE, J. E. S. Quart. J. Micr. Sci. (n.s.) 38 : 275-313. 1895.
50. MULLER, H. J. Science (n.s.), 66 : 84-87. 1927.
51. NACHTSCHEIM, H. Arch. Zellforsch. 11 : 169-241. 1913.
52. NEBEL, B. R. and RUTTLE, M. L. Z. Zellforsch. mikroskop. Anat. 26(2) : 281-292. 1937.
53. NICHOLS, M. L. Proc. Am. Phil. Soc. 41 : 77-112. 1902.
54. NICHOLS, M. L. J. Morphol. 20 : 461-478. 1909.
55. NONIDEZ, J. F. J. Morphol. 34 : 69-116. 1920.
56. PEACOCK, A. D. and SANDERSON, A. R. Proc. Second Intern. Congr. Sex Research, 239-247. 1930.
57. PROKOFIEWA, A. A. Arch. russ. anat. 10 : 64-79. 1931.
58. RABL, C. Morphol. Jahrb. 10 : 214-330. 1885.
59. ROBERTSON, W. R. B. J. Morphol. Physiol. 51(1) : 119-145. 1931.
60. SANDERSON, A. R. Genetica, 14 : 321-451. 1932.
61. SCHRADER, F. J. Morphol. 67 : 123-141. 1940.
62. SCHRADER, F. J. Morphol. 69 : 587-607. 1941.
63. SLACK, H. D. Proc. Roy. Soc. Edinburgh, 58 : 192-212. 1938.
64. SMITH, S. G. Sci. Agr. 21(5) : 245-305. 1941.
65. SMITH, S. G. and BOOTHROYD, E. R. Can. J. Research, C, 20(7) : 358-388. 1942.
66. STEVENS, N. M. Zool. Jahrb. 18 : 227-240. 1903.
67. STEVENS, N. M. J. Exptl. Zool. 5 : 359-374. 1908.
68. STEVENS, N. M. J. Exptl. Zool. 8 : 207-225. 1910.
69. STEVENS, N. M. Biol. Bull. 20 : 109-120. 1911.
70. STOCKING, R. J. Biol. Bull. 24 : 370-376. 1913.
71. SUTTON, W. S. Biol. Bull. 4 : 24-39. 1902.
72. TAUSON, A. Z. Zellforsch. mikroskop. Anat. 4(4) : 654-681. 1927.
73. TAYLOR, M. Quart. J. Micr. Sci. (n.s.) 60 : 377-398. 1914.
74. WHITE, M. J. D. Proc. Roy. Soc. (London) B, 125 : 516-523. 1938.
75. WHITING, P. W. J. Morphol. 28 : 523-577. 1917.
76. WILSON, E. B. J. Exptl. Zool. 13 : 345-450. 1912.

THE VITAMIN C (ASCORBIC ACID) CONTENT OF CANNED CHICKEN[1]

By E. J. Reedman[2], Leonard Buckby[3], and R. T. Legault[4]

Abstract

Vitamin C analyses on canned chicken indicate an appreciable amount in the product manufactured by improved methods. This amount, while low in proportion to that found in other foods, is sufficient to indicate the conservation of the vitamin by processes that minimize exposure to oxidation. The possible application of vitamin C analyses as an objective correlate to quality conservation in canning is discussed.

Introduction

Vitamin C is available in fresh and prepared fruits and vegetables, and also as synthetic *l*-ascorbic acid. Many additional foods contribute to the total received in average consumer diets. From a nutritional aspect a study of this factor in all foods, both raw and processed, is thus of value. Vitamin C is most unstable to oxidation, and to oxidation catalysed by heat or the presence of metals such as copper. Measurement of its retention during canning may indicate the degree to which oxidative changes in general have taken place. Thus, analysis for vitamin C may serve an additional purpose, as an index to quality preservation.

In conjunction with canning research on precooked poultry a number of analyses for ascorbic acid were conducted on both raw and processed products. Vitamin C studies reported herein are confined to canned precooked chicken, as the major marketed type of canned poultry. This commodity consists entirely of white and dark meat, and of broth prepared by cooking the whole cleaned and drawn birds, with head, feet, and all entrails removed.

Method

The method of analysis used throughout was the chemical titration procedure using 2-, 6-dichlorophenol indophenol as an oxidation–reduction indicator. The material was extracted with three separate portions of metaphosphoric acid using potassium cyanide as a stabilizer, and aliquots of the prepared extracts were titrated into the indophenol indicator, which was in turn standardized against pure synthetic *l*-ascorbic acid.

A number of concentrations of indicator were prepared in phosphate buffer at pH 7.2, and trial titrations on each extract enabled the choice of a concentration such that 1 ml. of indicator was decolorized to a decisive end-

[1] *Manuscript received May 1, 1942.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Published as Paper No. 77 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 1057.

[2] *Biochemist, Food Investigations.*

[3] *Laboratory Assistant.*

[4] *Laboratory Helper.*

point by 5 ml. or less of the extract under assay. A titration of an extract from a micro-burette into a receiving flask containing 1 ml. of the chosen indicator, diluted to a convenient volume with distilled water, took an average time of one minute. By titrating aliquots of extract in which the vitamin C had been destroyed by oxidation, it was ascertained that no reduction of the indicator by reducing substances other than the vitamin, occurred in the titration time used.

Reduced ascorbic acid was determined by direct titration; dehydroascorbic acid was converted to the reduced form by treatment of aliquots of the extracts with hydrogen sulphide. Trial aliquots were used to determine the time necessary for maximum conversion to the reduced form. All residual hydrogen sulphide was completely expelled with nitrogen gas prior to retitration for the estimation of the dehydro form of the vitamin. Combined ascorbic acid (2) was not present in the processed product assayed, since it would be hydrolysed from combination by the heat treatments used in canning.

Results

The results given in Table I show the various amounts of vitamin C in raw meat, in broth from precooking, and in the final can. The amount of vitamin C in raw lean meat was found to be too low to establish any significant difference between grades in raw dressed poultry. Dark muscle meat was, however, definitely higher than white muscle meat.

TABLE I

VITAMIN C IN RAW AND PROCESSED CHICKEN MEAT*

Type of product	Number of samples	Vitamin C, mg. per 100 gm.		
		Reduced ascorbic acid	Dehydro-ascorbic acid	Total
1. Raw lean, white muscle meat	8	0.6 – 0.9 (0.7)	0.1 – 0.3 (0.2)	0.9 – 1.1 (1.0)
2. Raw lean, dark muscle meat	8	1.0 – 2.5 (1.9)	0.5 – 0.9 (0.7)	1.9 – 3.0 (2.5)
3. Precooking broth, pressure method	8	9.8 – 12.5 (11.1)	1.5 – 2.5 (1.8)	11.4 – 14.8 (12.7)
4. Precooking broth, open method	8	1.5 – 3.6 (2.1)	1.5 – 1.6 (1.5) (2 only)	3.4 – 3.6 (3.5) (2 only)
5. Final whole can, open precooked	17	2.4 – 4.7 (3.3)	0.1 – 0.5 (0.3)	2.6 – 5.1 (3.6)
6. Final whole can, pressure precooked	13	3.0 – 7.0 (5.1)	0.1 – 0.6 (0.3)	3.6 – 7.1 (5.4)

* *Whole digits only are significant, but decimal places are given for purposes of comparison.*

In the preparation of canned poultry there are three heating treatments. The first of these is a precooking, to prepare the broth, reduce the time necessary in retorting, and to allow separation of meat and bones. The other two heat treatments involve some method of exhausting the residual air from the can, and the commercial sterilization or retorting of the sealed can. The last two treatments are fairly well standardized in commercial production. Precooking may result in loss of quality unless conservational methods are used, and this process seems most amenable to improvement in the canning of poultry.

The method of precooking most widely used commercially is that of heating the poultry in open steam-jacketed kettles. Since the maximum temperature that can be obtained is boiling point, the time required to precook is lengthy, and there is continued loss of flavour and oxidation. Pressure precooking, using closed steam-jacketed kettles, was introduced as a more efficient method for the commercial production of canned poultry. A small volume of water within the kettle generates steam from heat applied by the outer jacket, and steam pressures giving temperatures of 250° F. or even higher may be used for the product within the kettle. Pressure kettles enable a rapid precooking with maximum conservation of flavour and food value.

Table I illustrates the differences, per unit volume, in the vitamin C content of poultry broth from open and pressure precooking. While open-kettle broth is considerably more dilute than that produced by the pressure method, the observed differences cannot be accounted for on this basis, and must be due to actual loss by oxidation in the former method. In this study the same aluminum inset was used for cooking by each method, while the times and temperatures were those developed to obtain maximum quality in commercial practice. In many instances the vitamin C content of precooking broth is higher per unit than the raw meat itself. The heating treatment in steam or boiling water is an efficient extraction process, transferring to the broth water-soluble constituents from the whole drawn birds, including portions that are discarded as scrap in the packaging of the canned commodity.

Table II contains the results of analyses on finished cans of sliced chicken. These data, as well as those given for canned poultry in Table I, were obtained from products processed by exactly the same procedure with the exception of the method of precooking. Analyses are given for canned chicken produced from the different grades of raw dressed chicken, and for some commercial packs. In all cases the product had been stored for 10 months after processing.

It will be noted that there is a trend towards a higher vitamin C content in the canned product from birds of higher dressed quality. The canned chicken produced by pressure precooking is somewhat higher in vitamin C content than that produced by open precooking, using the same grade of raw meat. Extensive quality test panels have shown a superiority in the canned product from higher grades of raw material. These test panels, conducted on a consumer basis, definitely showed that pressure precooked product is superior to

TABLE II

VITAMIN C ANALYSIS OF CANNED POULTRY*

Sample	No. of cans	Reduced ascorbic acid, mg./100 gm.	Dehydro-ascorbic acid, mg./100 gm.	Total ascorbic acid, mg./100 gm.	Total ascorbic acid, mg./7 oz. can
A. Open precook					
1. Grade special, milk-fed	3	4.1 – 4.8 (4.3)	0.0 – 0.3 (0.1)	4.2 – 4.8 (4.4)	8.3 – 9.5 (8.9)
2. Grade A, milk-fed	2	4.3 – 5.2 (4.7)	0.1 – 0.8 (0.4)	4.4 – 6.0 (5.2)	8.7 – 11.9 (10.3)
3. Grade B, milk-fed	3	3.0 – 3.5 (3.3)	0.0 – 0.6 (0.2)	3.4 – 3.6 (3.5)	6.7 – 7.1 (6.9)
4. Grade B, Lot I	3	2.5 – 3.0 (2.6)	0.0 – 0.6 (0.2)	2.6 – 3.1 (3.2)	5.1 – 6.1 (5.7)
5. Grade B, Lot II	3	2.2 – 2.9 (2.5)	0.0 – 0.2 (0.1)	2.2 – 3.1 (2.6)	4.4 – 6.1 (4.8)
6. Grade C	3	2.1 – 2.9 (2.4)	0.2 – 1.0 (0.5)	2.6 – 3.2 (2.9)	5.1 – 6.3 (5.8)
B. Pressure precook					
7. Grade special, milk-fed	3	5.7 – 6.2 (5.9)	0.0 – 0.3 (0.2)	5.8 – 6.3 (6.1)	11.5 – 12.5 (12.1)
8. Grade A, milk-fed	3	5.0 – 7.0 (5.6)	0.1 – 0.2 (0.16)	5.2 – 7.1 (5.8)	10.3 – 14.1 (11.5)
9. Grade B, milk-fed	3	4.5 – 5.1 (4.8)	0.2 – 0.7 (0.4)	4.9 – 5.8 (5.2)	9.7 – 11.5 (10.4)
10. Grade B	3	4.5 – 5.6 (4.8)	0.2 – 0.7 (0.4)	4.7 – 6.1 (5.3)	9.3 – 12.1 (10.5)
11. Grade C	3	2.5 – 3.3 (3.0)	0.5 – 0.6 (0.56)	3.1 – 3.9 (3.6)	6.1 – 7.7 (7.1)
C. Commercial packs					
12. Special pressure precook	2	5.2 – 5.2 (5.2)	0.0 – 0.6 (0.3)	5.2 – 5.8 (5.5)	10.3 – 11.5 (10.9)
13. Special open precook	3	3.0 – 3.7 (3.3)	0.0	3.0 – 3.7 (3.3)	5.9 – 7.3 (6.6)
14. Commercial I	3	1.3 – 3.1 (2.0)	0.0 – 0.4 (0.2)	1.4 – 3.1 (2.2)	2.8 – 6.1 (4.3)
15. Commercial II	3	1.5 – 1.9 (1.7)	0.0 – 0.2 (0.06)	1.5 – 2.1 (1.8)	3.0 – 4.2 (3.6)
16. Commercial III	3	1.8 – 2.7 (2.2)	0.0 – 0.1 (0.03)	1.8 – 2.8 (2.3)	3.6 – 5.5 (4.5)
17. Commercial IV	3	1.3 – 1.4 (1.36)	0.0 – 0.3 (0.16)	1.4 – 1.6 (1.5)	2.8 – 3.2 (3.0)
18. Commercial V	3	1.5 – 2.0 (1.8)	0.0 – 0.4 (0.2)	1.7 – 2.3 (2.0)	3.4 – 4.0 (3.6)
19. Commercial VI	3	1.9 – 2.4 (2.1)	0.0 – 0.2 (0.06)	1.9 – 2.4 (2.2)	3.8 – 4.8 (4.4)

* *Statistical analysis of variance has shown a significant difference (necessary difference 0.9 mg.) between averages of grades as canned, and between canned product produced by pressure and open precooking. Both the latter are significantly higher than commercial canned precooked poultry.*

open precooked product, as judged by consumer preference. This preference was still apparent in packs showing no appreciable difference on chemical analysis for protein, fat, carbohydrates, and minerals.

Conclusions

The results of analyses show a higher content of vitamin C in canned poultry than in raw lean muscle meat, although only muscle meat is used in packaging. A considerable contribution to the total content comes from the broth prepared in precooking the whole drawn birds. The lower vitamin C content of sample cans from some commercial packs would indicate the need for more conservational processing.

Some correlation exists between quality and vitamin C conservation in canned chicken. The quality difference is not accountable for by ordinary chemical analysis, and the results offer a possible objective test for final quality based on vitamin C conservation. However, since the original vitamin C present in poultry meat is low, further detailed studies are planned on more suitable material in order that significant differences may be established throughout the entire canning processes used. Observations on vitamin C in the canning of fruit juices as made by Joslyn (1), indicate a close association of ascorbic acid and colour in citrus juices. Vitamin C analyses would appear to have practical application if a correlation between vitamin content and quality can be established in the food product concerned.

References

1. JOSLYN, M. A. Ind. Eng. Chem. Ind. Ed. 33(3) : 308-314. 1941.
2. REEDMAN, E. J. and MCHENRY, E. W. Biochem. J. 32 : 85-93. 1938.

A MATHEMATICAL ANALYSIS OF THE DISTRIBUTION IN MAIZE OF *HELIOTHIS ARMIGERA* Hb. (*OBSOLETA* F.)[1]

By Marjory G. Walker[2]

Abstract

The distribution among maize plants of the eggs of the American boll-worm, *Heliothis armigera* Hb., is discussed and analysed.

The problem is considered in relation to what is known of the connection between the state of development of maize plants and their attractiveness to ovipositing boll-worm moths. The actual frequency distribution of the eggs suggests a random as opposed to a uniform distribution, but it is shown that the conditions required for a pure mathematical random distribution cannot be satisfied. Because the maize plants differ from one another in absolute degree of attractiveness at any one time, and in relative degree of attractiveness with the passing of time, it is not true that every plant has the same chance of receiving any given egg.

It is demonstrated that a mathematical theory, which is eventually one of random distribution, but which incorporates a modification to allow for the varying degrees of attractiveness of the plants, gives a fairly good representation of the egg distribution found in the field.

Theoretical distributions to fit the data are calculated by two methods. One is a discontinuous process which is presented as only a rough approximation of what it is intended to express. The other uses the compound Poisson series of Greenwood and Yule. The continuous variation in nature both in space and time, which is the essential difficulty of the problem, is discussed.

Introduction

The problem of the distribution of animal populations is one that has been the subject of considerable discussion in recent years. The purely mathematical treatment of the question has outstripped the experimental verification of its theories and has, in consequence, forfeited the interest of the majority of biologists. Also, a plausible theory may be difficult or even impossible to verify, and until backed up by a suitable array of evidence it is at the mercy of any sceptic. The investigator who starts with facts is on surer ground, for then his hypotheses describe a series of possible events that *might* have produced the result actually observed. If the critic is now unable to improve matters by supplying a better idea, he must at least disprove the original assumptions, either by showing that they are not valid for an independent set of data, or by bringing forward evidence that has not previously been taken into consideration. In the present instance one of these possible objections has already been overcome because two separate sets of observations had been made on the same host-plant material. It was necessary, therefore, that the hypothesis developed in relation to one set should also be true for the other. This was found to be the case. Further, it was possible to interpret the

[1] *Manuscript originally received January 9, 1942, and in final form, May 14, 1942; submitted by Dr. W. R. Thompson, Imperial Parasite Service, Imperial Agricultural Bureaux, now stationed at Belleville, Ont.*

[2] *Formerly at Imperial Institute of Entomology, Farnham House Laboratory, Farnham Royal, Bucks, England.*

mathematical relation that was found to connect the two analyses in terms of what is actually known to happen in the field. There is thus some justification for this particular method of dealing with population problems.

The Problem and the Data

Marshall (2) published an account of the distribution and sampling of populations of the American boll-worm, *Heliothis armigera* Hb. (*obsoleta* F.). Because there is a high mortality among the larvae, particularly in the first and second instars, and because of their migratory movements, the most convenient indicator of the progress of an infestation has been found to be the oviposition of the moths. Parsons and Ullyett (3) had already shown that eggs laid by boll-worm moths are most unevenly distributed throughout a crop. Marshall's object was therefore primarily to determine the nature of the egg distribution and secondly to devise a method of sampling a crop so as to avoid inaccuracies due to the variation in the number of eggs. The present paper is a development of the first part of the problem[1].

Among the wide variety of crops attacked, maize is particularly favoured by the American boll-worm, oviposition beginning approximately with the appearance of the first tassels and reaching its peak by the time tassellation has occurred on all plants. Accordingly, for his experiment Marshall chose a field of drill-planted maize which was just coming into the attractive stage. A plot (23 × 24 sq. yd.) was taken at random in this field. As the maize was planted in rows a yard apart, there were in the plot 23 rows each 24 yd. long. The exact position of each of the 782 maize plants was measured and recorded in the form of a scale diagram. In Marshall's own words: "The experiment consisted of counting the eggs on every plant on two occasions with an interval of one week between the counts. The eggs of the moth are very minute, but the work was carried out by specially trained natives under white supervision with constant checking. The nature of the work makes a small error unavoidable, but this would certainly be no more than usually occurs in the course of the ordinary routine sampling. An individual count was conducted within the space of one day, and the eggs counted were crushed so that there could be no possibility of the second count being in error through the addition of old eggs." The two counts were recorded plant by plant. A summary of the result is given in Table I.

[1] *Note by Dr. W. R. Thompson.*

Dr. J. W. Hopkins of the Division of Applied Biology, National Research Laboratories, Ottawa, Canada, has very kindly pointed out that in 1938 a paper entitled "Discrete frequency distributions arising from several single probability values", was published in the Journal of the American Statistical Association, 33 : 390-398, by Dr. H. Muench. In this paper, Dr. Muench gave a solution, by the method of moments, of one of the hypotheses considered by Dr. Walker in connection with the oviposition of Heliothis, i.e., that there is a relatively small number of distinct categories of attractiveness, within each of which oviposition occurs at random, with a probability that is constant within each specific category but differs from one category to another. However, Dr. Walker's work had been completed and her paper prepared for publication before Dr. Muench's article appeared in print. She is now engaged in war work in Scotland and further revision of the paper at this time is impracticable.

TABLE I

No. of eggs per plant	No. of plants		No. of eggs per plant	No. of plants	
	1st Count	2nd Count		1st Count	2nd Count
0	206	101	17	2	11
1	143	72	18	1	7
2	128	89	19	–	1
3	107	84	20	–	3
4	71	66	21	–	1
5	36	40	22	–	2
6	32	56	23	–	2
7	17	43	24	–	1
8	14	49	25	–	–
9	7	28	26	–	1
10	7	39	27	–	1
11	2	13	28	–	–
12	3	17	29	–	2
13	3	12	30	–	–
14	1	14	32	–	1
15	1	12	34	–	3
16	1	10	51	–	1

Marshall says "The distribution for both counts was very similar. The biological aspect of the inquiry, as well as the nature of the distribution curves, suggested a Poisson series, but all attempts to fit such were unsuccessful. This might have been accounted for by the drawn-out tail of the curves where a small number of plants with high egg counts were recorded. Also the minuteness of the eggs and their often hidden position on the plant introduced a considerable error into the zero and few egg per plant classes. Such error would preclude any satisfactory fit." It is rather difficult to accept this explanation of a possible source of error in the egg counts. When an individual is set a task such as this one of counting eggs some of which are difficult to find, if he comes across a plant on which they are numerous he may quite easily lose count and record the number as too few or too many. On the other hand, if he is examining a plant but is unable to find any eggs on it, the fact that his job is to count eggs is a stimulus for him to keep on searching and to make sure of not missing any. From the psychological point of view, then, it is unlikely that errors of counting would be concentrated among the classes of plants with a few or no eggs, and in the present analysis it will be assumed that the counts were made with reasonable accuracy. On the other hand, the figures of the second count suggest the possibility of a bias in favour of even numbers. In Fig. 2 the graph of the data shows peaks at 6, 8, and 10 eggs per plant. Yule (4) discusses the idiosyncrasy in the case of reading scales. He points out that where a fraction of a unit has to be judged by eye, there is a tendency in some observers to round it off to 0.0 or 0.5 while in addition there is a preference for fractions expressed by an even digit as opposed to those expressed by an odd digit, with the exception of 0.5. Reading a scale is an entirely different proposition from that of searching for and counting objects. However a comparable feature in the collection of the

present data might be that a number of the searchers tended to be more satisfied that their efforts had been thorough and sufficient when they had found an even number of eggs on a plant than when they had found an odd number. In any case the biological evidence is that the eggs are laid singly and not in pairs. If there has been a particular bias in the recording of the numbers of the eggs in this investigation, it is begging the question to suppose that we can make any assessment of its effect. The recorded numbers of eggs are the results of a combination of the biological factors governing the distribution of the eggs, which is what we want to find out, and the human factors influencing the counting of the eggs. There are not sufficient data to allow the two to be dissociated. It can never be proved that the *recorded* distribution of the *Heliothis* eggs is other than what did in fact occur, and the present writer can only accept Marshall's statement that the observations were made with all due care and with constant checking. The assumption that the best fit for the peaks in question is a smooth curve running through them has been made independently of considerations of their possible cause.

As Marshall points out, the most likely biological hypothesis is that the boll-worm eggs would be distributed at random among the maize plants. The eggs are laid singly and are to be found in various parts of the plant. If it were known that the heavy mortality among the young larvae is due to overcrowding on heavily infested plants, then one would expect that the moths might show a tendency to oviposit to the advantage of their progeny, i.e., so that there would be signs of some attempt at a uniform distribution among the the plants. However, the records given above of the egg counts completely fail to suggest that any such faculty is put into use by the insects. If the boll-worm eggs are distributed among the maize plants at random in the strictly mathematical sense, then it must be true that the chance of any particular egg being laid on one specified plant is exactly the same as its chance of being laid on any *other* specified plant. If for any reason this condition is not quite satisfied, then obviously the frequency distribution of the eggs among the plants will not fit the calculated theoretical distribution. In the present paper a modification of the simple random distribution is suggested. This is based on the field observations of Parsons and Ullyett (3) and it is shown that with its aid theory can be made to fit facts.

The Method of Analysis

The object of the following illustration is to give the reader a clearer mental picture of random distribution, and particularly of the modifications that concern the present problem. Imagine an open oblong box. A person dropping marbles into it would probably tend to hold each marble somewhere over the middle of the box, but if he omitted this precaution, or better still, if he did not mind that some of the marbles missed the box altogether, then those marbles that fell into the box could be said to hit the bottom at random. Suppose that a hundred small boxes, in, say, 10 rows of 10, fitted exactly into the bottom of the large box, and that they were the same depth as their

container. If the small boxes were all of the same size, each box would have exactly the same chance, one in a hundred, of receiving any particular marble that fell within the big box. The marbles in the big box would then be distributed at random among the hundred little boxes. When the total number of marbles in the big box is known, it is easy to calculate the probable number of compartments that remain empty, or contain 1, 2, 3, etc. marbles.

Now suppose that the hundred small boxes, although all of the same height, are not all of the same size. They still fit into and exactly fill the bottom of the big box, but 25 of them are twice as large as the remaining 75. The marbles would still fall at random into the big box, and they would still *fall* at random into the hundred compartments of the box, but they would not be *received* at random by these compartments in the same sense as in the previous example. A *particular* large compartment is now twice as likely as a *particular* small compartment to receive any marble that falls into the big box. This complicates the calculation of the probable distribution of the marbles in the compartments. One method of overcoming the difficulty is to treat the distributions in the small and large compartments separately. It was postulated that the bottom of the large box was entirely covered by the 75 small compartments and the 25 larger compartments, each of the latter occupying double the basal area of the former. The area of the bottom of a small compartment being taken as a unit, there are 125 units in the area of the base of the large box of which 75, or three-fifths of the total are occupied by the small compartments, and 50, or two-fifths by the larger ones. It would therefore be expected that three-fifths of the marbles falling at random into the box would be received by the small compartments and two-fifths by the large compartments. When the total number of marbles in the box is known the two calculations required are the random distribution of three-fifths of that number among 75, and of two-fifths of the number among 25, from which the probable numbers of compartments with 0, 1, 2, 3, etc. marbles may be obtained as before. This, as will be explained later, corresponds in a simplified way to the first modification of random distribution required in the treatment of the present problem.

For the second modification imagine the large box as before with its hundred compartments all of the same size. After a certain fraction of the marbles have fallen at random into the box, suppose that 10 compartments, the end row for example, are removed and replaced by a fresh lot. Another fraction of the marbles falls into the box and this time the second row of compartments is renewed, a third fraction of marbles falls and then the third row is renewed, and so on. The marbles are all the time falling at random into the box, and they are in every case each being received by any one at random of a hundred similar compartments, though the individuals that comprise this total of a hundred change as time goes on. The calculations for the probable numbers of compartments with 0, 1, 2, 3, etc. marbles must be worked out each time 10 new compartments are substituted for 10 of the original ones. They are extremely laborious, but not more difficult mathematically than the essential random distribution calculation.

The hypothesis that is the basis of the explanation suggested for the distribution of *Heliothis* eggs among maize plants is that the changes that occur in the field as a crop matures could be likened to the theoretical cases just described.

Parsons and Ullyett (3) demonstrated, among other facts, the very great attractiveness of maize crops, at a certain rather limited phase of their development, to ovipositing *Heliothis* females. Here is what they observed at one farm: "There were four fields of maize which had been planted two or three weeks apart. The total acreages of maize and cotton were practically equal. Eggs of *H. obsoleta* were found first on the earliest crop of maize. At the time oviposition was first observed to occur on these maize plants, 10 per cent of them had just extruded the tip of the tassel or male inflorescence. The plant cotton at the time was flowering freely, and ratooned cotton bore fresh bolls and flowers in abundance. Cotton in this condition is considered to be highly attractive to *H. obsoleta*." Their data show, however, that "all the oviposition occurred in successive waves on the successive maize crops" (from mid-December till the beginning of March). "No *Heliothis* eggs were found on cotton on this farm until mid-March." Of another farm where there was one planting of maize and one main field of cotton they say:— "The maize crop was planted some weeks after cotton and experienced very difficult, low moisture conditions, in consequence of which growth was delayed and fruiting meagre. . . . . considerable oviposition was recorded on cotton before eggs were found on maize, but . . . . . when eggs appeared on the maize plants, egg-laying on cotton ceased and was not resumed until that on maize fell off. Thereafter renewed oviposition was recorded on cotton. In this instance it was observed again that *Heliothis* commenced laying on maize simultaneously with the appearance of the tassels. Although the maize plants were very poorly grown, they were not, apparently, unattractive to the bollworm moth. The maize and cotton lands were adjacent." On a third farm:— "The rows of maize were situated alongside the cotton. Records were taken in the strip of maize plants and the cotton concurrently. While oviposition was in progress on the maize, eggs were not found on the adjacent cotton. Immediately oviposition on the maize plants fell off, eggs were recorded on cotton . . . . . In this instance also an early and heavy oviposition had occurred on cotton prior to the commencement of recording and before the maize plants were in tassel as numbers of larvae were feeding on the cotton when observers first entered the crop."

"On three other farms similar conditions were observed. If maize was present as well as cotton, *Heliothis* at some period in the season practically confined oviposition to maize. From growth records it was observed that a definite relationship appeared to exist between egg deposition on maize and certain conditions in the reproductive phase of this plant."

As the quotations show, Parsons and Ullyett were very emphatic about "the coincidence between the appearance of eggs in numbers on maize and the production of the male inflorescence." This was because the general

implication of the earlier literature had been that the maize plants are most attractive to the moths during the production of the female flowers (silks) which appear after the extrusion of the tassels, or less precisely, that they are attractive from the time they are about 16 in. high until the leaves begin to turn yellow, a period of several months. To make their evidence more conclusive they made daily counts of eggs on a number of marked maize plants, which were examined twice weekly for height and for the development of leaves, tassels, and silks. The data, presented in an interesting diagram, show the striking correlation between oviposition and tassellation. "Only a very few eggs were found before the first tassels appeared. Thereafter eggs became abundant, and the peak of the oviposition coincided with tassellation on all plants. Up to that time 70 per cent of the total eggs laid had been recorded." "During the main period of abundant, fresh silk production, egg-laying did not occur."

"This coincidence of insect–plant behaviour is being investigated at the present time with regard to certain chemical changes in the plant which are associated with the reproductive phase."

As has already been pointed out, oviposition by *Heliothis* among the maize plants can be strictly random only if every plant has the same chance of receiving any particular egg, but, as the field evidence has shown over and over again, oviposition is associated with the appearance of the tassels. Now, in the diagram already mentioned, it can be seen that among the plants specially observed by Parsons and Ullyett, the first appearance of tassels continued in the different plants over a period of 13 days. It would thus seem to be a reasonable hypothesis that the plants are not all equally attractive at any one time. If this idea is accepted it must be at once obvious that the arrangements of eggs among the plants cannot be comparable to the elementary random distribution. This brings us to the application of the first of the modifications explained by the example of marbles dropping into compartments. When some compartments are larger than others, they are more likely to receive marbles. In the same way the maize plants that protrude their tassels earliest may, to begin with, be more attractive than the other plants. If Parsons and Ullyett are right when they suggest that the attractiveness of maize to *Heliothis* is due to chemical changes that take place in the plants at a certain phase in their development, then it is the early development of individual plants and not the crop as a whole that is responsible for the commencement of oviposition in a maize field and the corresponding cessation in a neighbouring cotton field.

Now although Parsons and Ullyett kept a record of the development of the individual plants on which they made the daily egg counts, they do not mention that they found more eggs on plants in some particular stage of tassel development than on those that were less mature at the time. But supposing that out of a hundred maize plants, 50 of them, being at a more attractive stage, were twice as attractive as the remaining 50. Then on an analogy with the 50 large and 50 small compartments, if a total of 600 eggs were laid, the

50 attractive plants should receive 400 eggs and the others only 200 eggs. But if the 400 eggs were distributed at random among the 50 plants—and there is no obvious reason why they should not be—then according to the mathematical calculation we should expect that some of these plants would receive quite small numbers of eggs, as well as others receiving large numbers. In the same way in the distribution at random of 200 eggs among 50 plants, a certain proportion of the plants would probably receive quite large numbers of eggs. This overlapping of the two distributions would tend to obscure the law that plants at a certain stage of maturity attain their maximum phase of attractiveness and receive *on the average* the greatest number of eggs from the ovipositing moths.

There were 782 maize plants in Marshall's experimental plot. The first count of the *Heliothis* eggs gave a total of 1934, an average of 2.47 eggs per plant. As Marshall pointed out, the egg frequency distribution of the field data differs from the theoretical distribution resulting from the calculation of the random distribution at the rate of 247 eggs per 100 plants, in having too many plants with no eggs and with very few eggs, and again too many plants with high numbers of eggs. After a number of trials it was found that on the assumption that $\frac{4}{7}$ of the plants received eggs at random at the average of 247 eggs per 100 plants (the average for the whole plot) that $\frac{2}{7}$ of the plants received eggs at random at the average of 30 eggs per hundred plants, while the remaining $\frac{1}{7}$ of the plants received at random an average of 681 eggs per 100 plants, the theoretical egg distribution in the plants taken as one group bears a satisfactory resemblance to the real distribution. The second, third, and fourth columns of Table II show the random distributions of 30, 247, 681 respectively in 100, and the fifth column shows the theoretical egg frequencies among the plants when 247 eggs are distributed per 100 plants in the manner outlined. This column is calculated from the other three columns line by line and it may be represented by the general formula:

$$\{\tfrac{2}{7} \times (R.D.\text{ of 30 in 100})\} + \{\tfrac{4}{7} \times (R.D.\text{ of 247 in 100})\} + \{\tfrac{1}{7} \times (R.D.\text{ of 681 in 100})\},$$

where *R.D.* stands for Random Distribution and is calculated from the terms of the Poisson distribution, namely

$$Ne^{-m}\left(1 + m + \frac{m^2}{\underline{|2}} + \frac{m^3}{\underline{|3}} + \ldots\ldots\right),$$

where $N = 100$, the number of plants and m, the hypothetical arithmetic mean of the numbers of eggs per plant in the various groups, has the values 0.30, 2.47, and 6.81. The theory underlying the use of this formula is given in most textbooks on statistics; e.g. Yule and Kendall (5). In Fig. 1, also, the actual and theoretical distributions of the eggs are shown, the scale being adjusted to represent the total 782 plants. It differs from Table II in that the three component curves that represent the egg distribution in the three grades of plants are shown in the same relative scale, and the sums of their ordinates give the ordinates of the curve for the whole population.

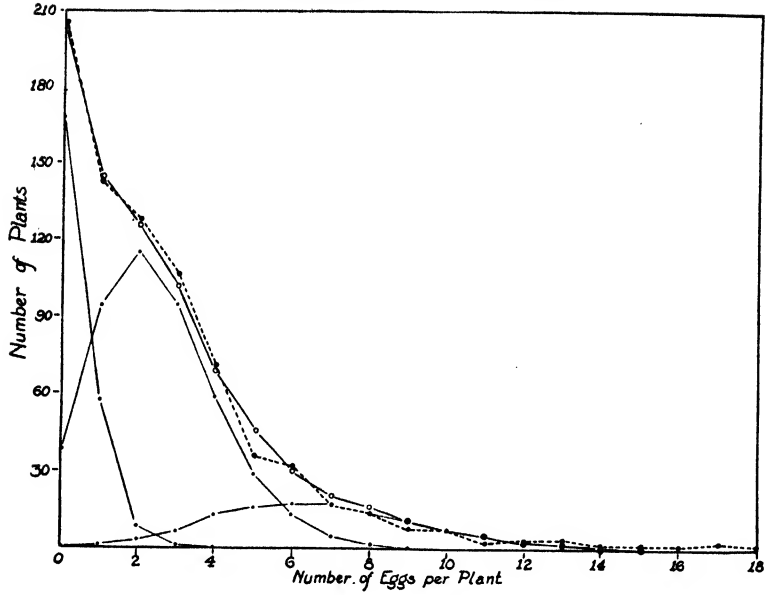


FIG. 1. *First egg count and theoretical distribution obtained by summing three random distribution series;* ●- - - -● = *data,* ○——○ = *theoretical distribution,* ●——● = *component curves.*

TABLE II

No. of eggs per plant	Random distributions in 100			Theoretical distribution	1st Count per 100 plants
	30	247	681		
0	74.082	8.458	0.110	26.02	26.34
1	22.225	20.892	0.751	18.40	18.29
2	3.337	25.802	2.557	16.06	16.37
3	0.334	21.243	5.804	13.06	13.68
4	0.025	13.118	9.882	8.92	9.08
5		6.480	13.459	5.63	4.60
6		2.668	15.276	3.71	4.09
7		0.941	14.861	2.66	2.17
8		0.291	12.651	1.97	1.79
9		0.080	9.572	1.41	0.90
10		0.020	6.519	0.94	0.90
11		0.004	4.036	0.58	0.26
12			2.290	0.33	0.38
13			1.200	0.17	0.38
14			0.584	0.08	0.13
15			0.265	0.04	0.13
16			0.113	0.02	0.13
17			0.045	0.01	0.26
18			0.017		0.13
19			0.006		
20			0.001		

The second count was made a week after the first, and, as has been mentioned the numbers of eggs recorded were quite independent of those in the first count. On this occasion 4499 eggs were present on the 782 plants making an average of 5.75 eggs per plant. The curve formed by the egg distribution (Fig. 2) is rather irregular, but a fairly good fit was obtained from the assumption that $\frac{5}{14}$ of the plants received eggs at random at the average of 746 eggs per 100 plants, $\frac{5}{14}$ received eggs at random at the average of 247 eggs per 100 plants, $\frac{1}{7}$ received eggs at random at the averages of each of 50 and 1492 eggs per 100 plants. Table III shows each of these distributions and also the distribution for the whole population calculated from the formula:

$$\{\tfrac{1}{7} \times (R.D. \text{ of } 50 \text{ in } 100)\} + \{\tfrac{5}{14} \times (R.D. \text{ of } 247 \text{ in } 100)\} + \{\tfrac{5}{14} \times (R.D. \text{ of } 746 \text{ in } 100)\} + \{\tfrac{1}{7} \times (R.D. \text{ of } 1492 \text{ in } 100)\}.$$

The four component curves are shown in scale in Fig. 2. No theoretical curve based on hypotheses of random distribution can be constructed to follow the sharp angles of the data curve. The one given does, however, represent quite well a smoothed curve of the data.

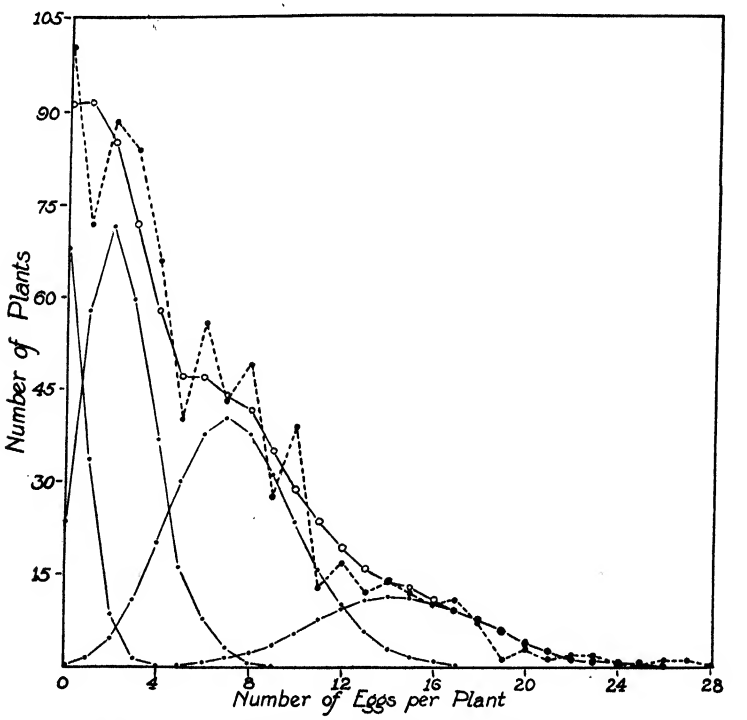


FIG. 2. *Second egg count and theoretical distribution obtained by summing four random distribution series;* ●- - - -● = *data,* O——O = *theoretical distribution,* ●——● = *component curves.*

TABLE III

No. of eggs per plant	Random distributions in 100				Theoretical distribution	2nd Count per 100 plants
	50	247	746	1492		
0	60.665	8.458	0.058	0.000	11.71	12.92
1	30.326	20.892	0.429	0.000	11.95	9.21
2	7.582	25.802	1.602	0.004	10.92	11.38
3	1.264	21.243	3.983	0.018	9.19	10.74
4	0.158	13.118	7.429	0.068	7.37	8.44
5	0.016	6.480	11.083	0.204	6.30	5.11
6	0.001	2.668	13.780	0.507	5.96	7.16
7		0.941	14.686	1.082	5.74	5.50
8		0.291	13.695	2.018	5.28	6.27
9		0.080	11.351	3.345	4.56	3.58
10		0.020	8.468	4.991	3.74	4.99
11		0.004	5.743	6.771	3.02	1.66
12			3.570	8.418	2.48	2.17
13			2.049	9.661	2.11	1.53
14			1.092	10.296	1.86	1.79
15			0.543	10.242	1.66	1.53
16			0.253	9.550	1.45	1.28
17			0.111	8.381	1.24	1.41
18			0.046	6.948	1.01	0.89
19			0.018	5.456	0.79	0.13
20			0.007	4.070	0.58	0.38
21			0.002	2.892	0.41	0.13
22			0.001	1.961	0.28	0.26
23				1.272	0.18	0.26
24				0.791	0.11	0.13
25				0.472	0.07	—
26				0.271	0.04	0.13
27				0.150	0.02	0.13
28				0.080	0.01	—
29				0.041		0.26
30				0.020		—
31				0.010		0.26
32				0.005		—
33				0.002		—
34				0.001		0.38
51						0.13

Having given a possible explanation of the egg distribution found in the first and second examinations of the maize plants we should now be in a position to discuss the sum of the two distributions in terms of the same theory. Marshall dealt with the resemblances and differences between the two egg counts but not with their sum. However, since his records preserved the individuality of each plant it was a simple matter to add together the numbers of eggs found on the two occasions and so to obtain the frequency distribution for the grand total of eggs observed (Table IV, final column). This could have been dealt with in the same manner as were the two previous distributions, by dividing the number of plants into groups representing different degrees of attractiveness, and working out a new series of random distributions to correspond. But since the real distribution of the sum was obtained from two real distributions it is more satisfactory that the theoretical distribution for the sum should also be derived from the two theoretical distri-

TABLE IV

No. of eggs per plant	Random distributions									Theoretical distribution	Sum of two counts
	$\frac{3}{11}$ at 80 per 100	$\frac{3}{11}$ at 277 per 100	$\frac{3}{11}$ at 494 per 100	$\frac{3}{11}$ at 731 per 100	$\frac{3}{11}$ at 776 per 100	$\frac{3}{11}$ at 993 per 100	$\frac{3}{11}$ at 1427 per 100	$\frac{3}{11}$ at 1739 per 100	$\frac{3}{11}$ at 2173 per 100		
0	37.65	3.50	1.60	0.02	0.04	0.01				42.82	45
1	30.12	9.69	7.90	0.14	0.28	0.07				48.20	54
2	12.05	13.43	19.50	0.50	1.08	0.34				46.90	50
3	3.21	12.40	32.12	1.22	2.78	1.11	0.02			52.86	54
4	0.64	8.59	39.66	2.22	5.40	2.75	0.06	0.01		59.33	61
5	0.10	4.76	39.19	3.25	8.38	5.47	0.17	0.03		61.35	68
6	0.01	2.20	32.27	3.96	10.84	9.05	0.42	0.09		58.84	53
7		0.87	22.77	4.13	12.02	12.84	0.85	0.22		53.70	50
8		0.30	14.06	3.78	11.66	15.94	1.51	0.49	0.01	47.75	39
9		0.09	7.72	3.06	10.05	17.59	2.40	0.94	0.03	41.88	46
10		0.03	3.81	2.24	7.80	17.47	3.42	1.64	0.07	36.48	31
11		0.01	1.71	1.49	5.50	15.77	4.44	2.59	0.13	31.64	26
12			0.70	0.91	3.56	13.05	5.28	3.75	0.24	27.49	26
13			0.27	0.51	2.12	9.97	5.79	5.02	0.39	24.07	36
14			0.10	0.27	1.18	7.07	5.91	6.24	0.61	21.38	27
15			0.03	0.14	0.60	4.68	5.62	7.23	0.89	19.19	15
16			0.01	0.07	0.29	2.90	5.01	7.85	1.20	17.33	16
17				0.03	0.13	1.70	4.21	8.03	1.54	15.64	8
18				0.01	0.06	0.94	3.33	7.76	1.86	13.96	13
19					0.02	0.49	2.50	7.11	2.13	12.25	15
20					0.01	0.25	1.79	6.18	2.31	10.54	9
21						0.12	1.21	5.12	2.39	8.84	7
22						0.05	0.79	4.05	2.36	7.23	4
23						0.02	0.49	3.05	2.23	5.79	5
24						0.01	0.29	2.21	2.02	4.53	3
25							0.17	1.54	1.76	3.47	4
26							0.09	1.03	1.47	2.59	8
27							0.05	0.66	1.18	1.89	—
28							0.02	0.41	0.92	1.35	—
29							0.01	0.25	0.69	0.95	1
30								0.14	0.50	0.64	—
31								0.08	0.34	0.42	1
32								0.04	0.24	0.28	1
33								0.02	0.16	0.18	1
34								0.01	0.10	0.11	1
35									0.06	0.06	—
36									0.04	0.04	1
37									0.02	0.02	—
38									0.01	0.01	—
39											—
40											—
42											1
48											1
52											1

butions. The fact of this being possible is an additional justification for the hypotheses used.

Marshall says: "*A priori* it was to be expected that over a short period of time, as between the two counts taken in this experiment, that the crop should retain its inherent differences in growth and attractiveness." One would agree that if, on a certain date, some plants were more advanced and some

less advanced than others, then it is to be expected that a few days later they would still be in the same *relative states of development;* but an opinion as to their expected *relative states of attractiveness* on the two occasions would need to be based on a more precise knowledge of what causes maize plants to become attractive to *Heliothis*. Referring to the arrangement of the plants in rows and to a method of assessing the egg distributions by taking the total number of eggs for each individual yard length of the rows, Marshall continues: "A correlation trial was worked out between the first count per yard and the second count on the same yard. (All yard lengths having no plants in them were removed from the analysis.) A significant correlation coefficient was found of +0.3348 with 454 degrees of freedom. This was highly significant at the 0.01 level, and the validity of the correlation was established. A similar correlation on egg counts *per plant* on the two occasions also showed a positive correlation between the two counts. It was therefore definitely established that the same portions of the crop tended to retain their relative attraction to the boll-worm moth over short periods of time".

In the present paper the main assumption underlying the analysis is that the plants are not all equally attractive to the moths. The basis for this assumption is the evidence of Parsons and Ullyett that oviposition starts when the first plants begin to show the tassels, and continues at a rapidly *increasing rate* as more and more plants show their tassels. The maximum number of eggs is present at the time when all the plants have protruded tassels, but the *rate* of increase in the number of eggs begins to *diminish* some days earlier at the period when the proportion of plants with fully extruded tassels is showing a rapid increase. The natural inference is that the first plants to have their tassels full out are those that originally attracted the first moths to the field. The first plants to be most attractive would be the first to pass the period of greatest attractiveness, and hence would be responsible for the falling off in the rate of increase in the number of eggs. The tendency for a positive correlation between egg counts on individual plants at an interval of as much as a week would therefore seem to depend on the relation of the time when the first count was made to the whole period of oviposition. If the first count were made early in the oviposition period, the second count, a week later, would be made while all the plants were still, on the whole, increasing in attractiveness. On the other hand, if the first count were made just before the peak of oviposition, when none, or only a few of the plants had passed out of the most attractive stage, then a week later a much higher proportion of plants would have passed from the most attractive to a less attractive stage. In a case such as the latter the correlation between the two egg counts, if still positive, would certainly be less significant than in a case of the former type.

The theoretical distribution for the sum of the two counts was calculated first of all on the hypothesis that the plants that were very attractive in the first count were still the most attractive in the second count. The basic random distributions that had to be worked out were therefore as follows:

Fraction of population	No. of eggs received per 100 plants		
	1st Count	2nd Count	Total
$\frac{1}{16}$	30	50	80
$\frac{2}{16}$	30	247	277
$\frac{8}{16}$	247	247	494
$\frac{5}{16}$	247	746	993
$\frac{2}{16}$	681	1492	2173

Fig. 3 shows these five random distributions and the curve which is the sum of their ordinates. In comparison with the real distribution it is seen to have too high proportions of plants in the groups with very many and very few eggs. After several trial rearrangements the best fit was obtained from the one given below:

Fraction of population	No. of eggs received per 100 plants		
	1st Count	2nd Count	Total
$\frac{1}{16}$	30	50	80
$\frac{2}{16}$	30	247	277
$\frac{3}{16}$	30	746	776
$\frac{8}{16}$	247	247	494
$\frac{5}{16}$	247	746	993
$\frac{3}{16}$	247	1492	1739
$\frac{2}{16}$	681	1492	2173
$\frac{2}{16}$	681	746	1427
$\frac{1}{16}$	681	50	731

The details of these nine random distributions are given in Table IV where, for convenience, they have been worked out to the requisite fractions of 782, the total plant population of the sample plot. The theoretical curve in Fig. 4, while not a particularly good fit, is better than that in Fig. 3, and, considering the irregularities of the real curve and the limitations of the method of calculation, it is probably as much as could be expected from the nature of the hypotheses.

The most important features of the curve in Fig. 4 are, firstly, that the group of plants that is most attractive in the second egg count includes only one-fourth of those that were most attractive in the first count, the remainder being derived from those that were only moderately attractive; secondly, the remainder of the highly attractive group in the first count is considered to have passed the peak of attractiveness so that its total of eggs recorded in the second count is considerably less than the maximum. In particular one-fourth of the group is supposed to have passed almost entirely out of the attractive phase, while the remaining half of the group is still quite strongly attractive. "Group" is here used in connection with the mathematical and not the spatial aspect of the problem. This is the second modi-

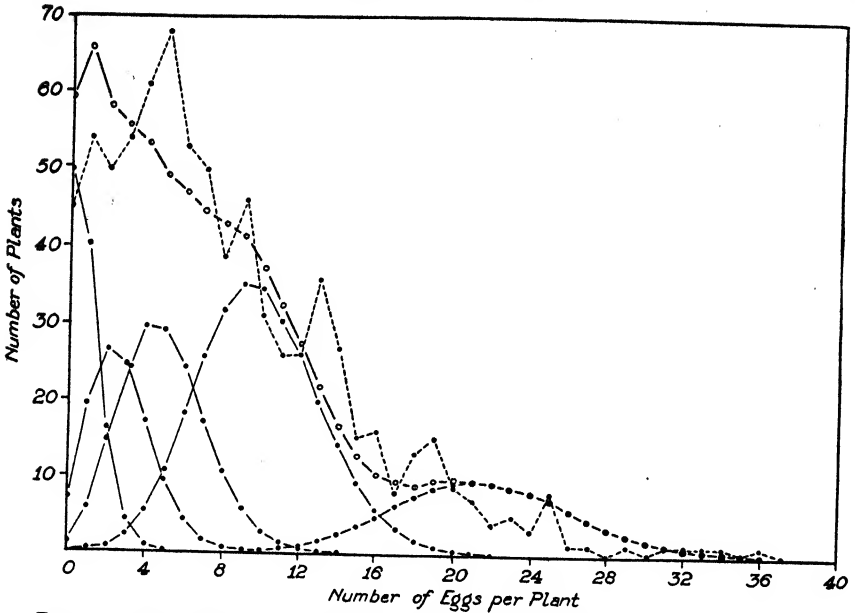


FIG. 3. *Sum of the two egg counts and a theoretical distribution based on the assumption that the plants have retained, more or less, the same relative attractiveness to the moths at both periods;* ●- - - -● = *data,* ○———○ = *theoretical distribution,* ●———● = *component curves.*

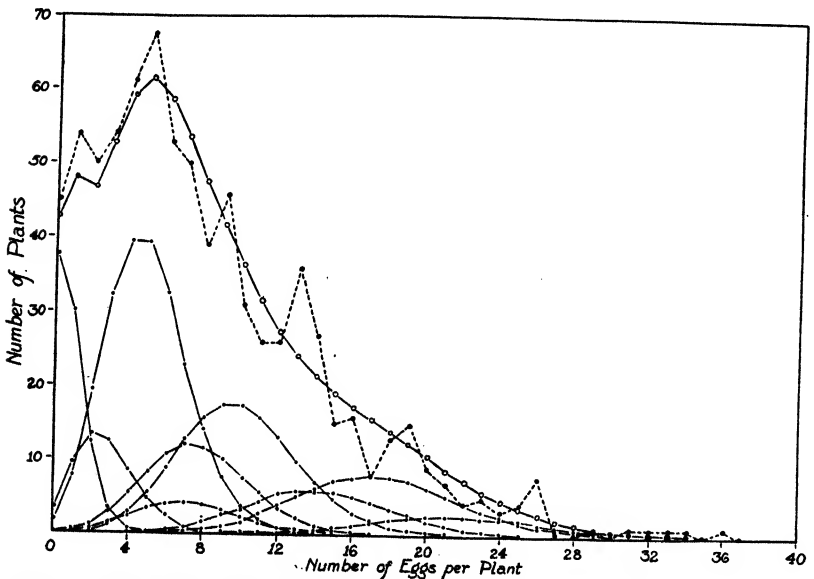


FIG. 4. *Sum of the two egg counts and an improved theoretical distribution. In this case it is assumed that the plants, by their development in the interval between the two counts, have altered in their relative attractiveness to the moths;* ●- - - -● = *data,* ○———○ = *theoretical distribution,* ●———● = *component curves.*

fication of sample random distribution which was illustrated by the theoretical case of dropping balls into boxes. A set of boxes represents a particular group of plants, the most attractive plants, for example, or the least attractive. As the balls drop some boxes are added to the group, others are removed. So with the plants, as time passes, new plants reach the most attractive phase while others continue their development beyond that phase and become classified along with less attractive plants in another group.

The various groups or types of plants are considered to be mixed indiscriminately throughout the plot. In the first egg count, a large fraction of the plants, $\frac{2}{7}$, was considered to be attractive in only a very slight degree. During the course of the week it would be expected that these plants would have developed and become more to the liking of the moths. The interpretation, according to the accepted calculation for the sum of the counts, is that three-eighths of that group ($\frac{3}{2\,8}$ of the population of the plot) was still almost entirely neglected by the moths, in spite of the greatly increased rate of oviposition. This could be due to the relative backwardness of the plants, or because some or all of them were in an unhealthy condition from some other causes. The remainder of this group is assumed to have developed normally according to expectation, such that one-quarter became moderately attractive and the other three-eighths more so. A similar fate in the second count is shown for the large group of plants that were moderately attractive in the first count. To avoid confusion it should be remembered that the progress of tassellation in the field as a whole may safely be assumed to have attracted more and more moths to that field. There was thus a much higher rate of egg-laying during the week between the counts than during the week previous to the first count, and therefore the numbers of eggs allocated to the group of plants designated as moderately attractive, most attractive, etc., were proportionately very much higher in the second count than in the first.

The Limitations of the Method

In the preceding section, field data on the distribution of *Heliothis* eggs among maize plants have been analysed with the aim of explaining how that particular numerical arrangement could have come about. The explanation was based on recorded observations on the relation between the development of maize plants and their attractiveness to ovipositing *Heliothis* moths. The original and most simple theory was that the female moths distributed their eggs at random among the plants, and Marshall tested the idea by comparing his data with a random distribution calculated from a Poisson series. The reason why this theory failed to agree with the facts was quite plain from the work of Parsons and Ullyett. These workers showed that not only was it true in the general sense, that a maize crop becomes attractive to the moths when the tassels are beginning to appear, but that more particularly the increasing degree of oviposition can be correlated with the rising percentage of tassels that appear, and further that the percentages of tassels fully developed and of silks showing are associated with the subsequent dwindling of

the oviposition rate in the crop. This rising and falling in the intensity of oviposition does not correspond to the normal egg-laying cycle found in the cases of insects with isolated generations all the individuals of which reach the same stage of development at approximately the same time. The *Heliothis* moths oviposit in other crops before and after a maize crop is in the desired stage of maturity, and so the peak of egg-laying activity in a particular field of maize represents the time when the greatest numbers of moths are present. If it had been obvious to Parsons and Ullyett from their daily examinations of the individual plants that the moths showed a special preference for a particular stage of development, they would undoubtedly have stressed the point in more detail than by merely discussing the trend of oviposition in its relation to the crop as a whole. Since the distribution of eggs among the plants certainly does not suggest uniformity, one therefore naturally returns to the idea of a random distribution, adjusted to include the concept of the non-uniformity of the plants. It has already been pointed out that in the case of a random distribution, modifications such as those adopted in the present analysis, could quite easily mask the fact that on any given day during the period of oviposition plants in a certain state of development are tending on the whole to receive more eggs than the other plants.

Marshall's data consisted of the numbers of eggs on each of several hundred maize plants, recorded on two separate occasions with an interval of a week. The eggs take about four days to hatch, so that each count gives the sum of the eggs laid on a plant during a period of four days. In the whole experimental plot the total for the second count was more than 2.5 times as great as the total for the first count. In other words we might say that during the second period of four days the plot was on the whole 2.5 times as attractive as it was during the first four days. But what the present data do not show is the day to day development of this attractiveness. Again, for the calculation of the theoretical curve to correspond with each of the egg counts it was necessary to assume that the plants were divided into certain arbitrary numerical groups. For example, in the first distribution, $\frac{4}{7}$ of the plants were assumed to receive an average number of eggs equal to the average of the whole population, but a further $\frac{1}{7}$ of them received an average of nearly three times as many eggs, while the average for the remainder was only about 0.1 times that of the population. But even if, in fact, the plants could be classified in these three grades of average attractiveness, which would be expressed in terms of average development, this would be only a crude representation of what was really happening in the field.

The processes of development are continuous. Any one plant passes through an infinite number of developmental stages, and at any one instant the plants in a field exhibit, within the limits defined by the most advanced and the least advanced plant, a very large number of stages of this same development. In time, and to a certain extent, in space also, the natural phenomena show continuous variation, but the mathematical method employed has dealt with this feature to a very limited extent. The plants were allocated

to groups, the averages for each of the groups being widely different from each other. Yet the plants within each group were treated mathematically as if they were all attractive to exactly the same degree. The building up of the egg distribution curve from three or four small curves was an attempt to get nearer to a picture of what one imagined was a possibility in the field. To return to the analogy of the marbles dropping into boxes, the idea of the compartments being of different sizes represents the plants in different stages of attractiveness. We could, if necessary, deal with as many sizes of compartments as we liked in calculating the random distributions. This would correspond with the range in attractiveness of the plants on any one day. They also vary in relation to one another with the passing of time. Because the data in the present problem represent the oviposition for two separate periods of four days, the relation between the plants on the two occasions could be represented only by a displacement in the fitting together of the two sets of theoretical curves—*vide* the text data referring to Figs. 3 and 4. A more exact mathematical treatment of a problem of this kind would need, as a basis, a concept like the one in which marbles were supposed to drop at random into the compartments, but certain sets of these compartments were replaced by new ones from time to time. In the account given earlier in the paper, a step by step substitution of the 100 compartments in lots of 10 was described. But the substitution could be imagined to take place in a less disjointed manner and at a varying rate, so that the total number of compartments in use is not necessarily constant. In this way the compartments that were actually receiving marbles at any instant could correspond to the maize plants, which were, for example, the most attractive during any given day, but the calculation of a random distribution among all those compartments would present some difficulty.

The Greenwood–Yule Distribution

A mathematical investigation of a similar problem embodying this concept of continuous variation was made by Greenwood and Yule (1). The practical application of their work was the analysis of data derived from certain records including those of the numbers of accidents occurring in groups of factory workers and the frequency distribution of those accidents among the individuals of the groups. It was readily shown that accidents did not happen altogether at random among the members of the several groups of workers which is no less than we would expect from a brief consideration of the great differences that exist between one man and the next. The theoretical random distribution as represented by the simple Poisson series therefore did not fit the accident frequency distribution. This led to an examination of modifications of the random distribution theory including what is described as the "infinitely compound Poisson distribution". The authors say— "We now suppose that the population at risk consists of persons (or other variates) the liabilities of whom to accident vary, the frequencies being assigned by the ordinates of $f(\gamma)$) where γ is a variable parameter." It was first of all assumed that $f(\gamma)$ is a normal function, but this hypothesis was discarded

for two reasons, namely, that the resulting compound Poisson series was rather unmanageable and that "values of γ may be supposed to range from zero far in the positive direction so that $f(\gamma)$ should be skew. A choice of skew curves is arbitrary." The form actually adopted is the curve corresponding to the skew binomial. It is a Pearson Type III curve and has the equation

$$y = y_0 e^{-c\gamma}\gamma^{r-1}.$$

When it is integrated in conjunction with the terms of the series representing simple random distribution, viz.

$$e^{-\gamma}\left(1 + \gamma + \frac{\gamma^2}{\underline{|2}} + \frac{\gamma^3}{\underline{|3}} + \ldots\ldots\right)$$

the following series results

$$\left(\frac{c}{c+1}\right)^r\left(1 + \frac{r}{c+1} + \frac{r(r+1)}{\underline{|2}\,(c+1)^2} + \frac{r\,(r+1)(r+2)}{\underline{|3}\,(c+1)^3} + \ldots\ldots\right).$$

The values of the mean and the second moment are respectively $\frac{r}{c}$ and $\frac{r(c+1)}{c^2}$.

The distribution is therefore easily fitted from the mean and second moment of the observations.

Before discussing the applicability of the hypothesis of the Greenwood–Yule series to the problem of the distribution of *Heliothis* eggs we shall compare the observations with the theoretical distributions. In the three cases the constants '*r*' and '*c*' are found to have the following values.

	1st Count	2nd Count	Sum of counts
c	0.50298	0.21873	0.21834
r	1.24392	1.25840	1.79613

The respective distributions are shown in comparison with the data in Table V, where the numbers underlined indicate that the frequencies in the remaining classes have been summed. They are illustrated in Figs. 5, 6, and 7. In the case of the first egg count the Greenwood–Yule distribution in Fig. 5 is obviously not such a good fit for the data as the theoretical distribution in Fig. 1. The irregularities of the second count prevent a really good fit and there is apparently little to choose between the two distributions that have been selected to represent it. We might attempt to lessen the effect of the irregularities in question by making assumptions as to how they may have arisen. But that would involve further assumptions of what theoretically the nature of the distribution ought to be. Such hypotheses could not be justified, for the aim of the investigation is to discover what the distribution actually is. To state what it should be, would be to reason in a circle. Whatever we may suspect about the accuracy of the observations, we are not, at this stage, entitled to challenge them on theoretical grounds, and we must accept Marshall's statement that they were made, under his supervision, with all due care, and with adequate checking. One point of interest in the comparison between the Fig. 2 distribution and the Greenwood–Yule distribution

TABLE V

No. of eggs per plant	1st Count		2nd Count		Sum of two counts	
	Data	Greenwood–Yule distribution	Data	Greenwood–Yule distribution	Data	Greenwood–Yule distribution
0	206	200.4	101	90.0	45	35.7
1	143	165.8	72	92.9	54	52.6
2	128	123.8	89	86.1	50	60.3
3	107	89.1	84	76.8	54	62.6
4	71	62.9	66	67.1	61	61.7
5	36	43.9	40	57.9	68	58.7
6	32	30.4	56	49.5	53	54.5
7	17	20.9	43	42.1	50	49.9
8	14	14.3	49	35.7	39	45.0
9	7	9.8	28	30.1	46	40.2
10	7	6.7	39	25.4	31	35.6
11	2	4.5	13	21.3	26	31.4
12	3	3.1	17	17.9	26	27.4
13	3	2.1	12	14.9	36	23.9
14	1	1.4	14	12.5	27	20.7
15	1	1.0	12	10.4	15	17.9
16	4	1.9	10	8.7	16	15.4
17			11	7.2	8	13.3
18			7	6.0	13	11.4
19			1	5.0	15	9.7
20			3	4.2	9	8.3
21			1	3.5	7	7.1
22			2	2.9	4	6.0
23			2	2.4	5	5.1
24			1	2.0	3	4.3
25			0	1.6	4	3.7
26			1	1.4	8	3.1
27			1	1.1	0	2.6
28			0	1.0	0	2.2
29			7	4.4	1	1.9
30					0	1.6
31					1	1.3
32					1	1.1
33					1	0.9
34					5	4.9

in Fig. 6, is that the former seems to be a better representation of the middle section of data curve, while the latter is distinctly superior for the section to the right of the curve. In fact in each of these cases the Greenwood–Yule distribution gives a better representation of the numbers of plants with high numbers of eggs. The reason for this will be found in an examination of the basic hypotheses of the Greenwood–Yule distribution. It assumes that the plants vary in their attractiveness to the ovipositing moths, and this variation is expressed by an equation which states that a diminishing fraction of the plants may be increasingly attractive with a limit at infinity. The relation between the fraction of plants and the degree of attractiveness is variable in that the fraction may diminish more or less rapidly, although the limit of attractiveness is theoretically never less than infinitely great. Because the mathematical constants of the Greenwood–Yule distribution are derived

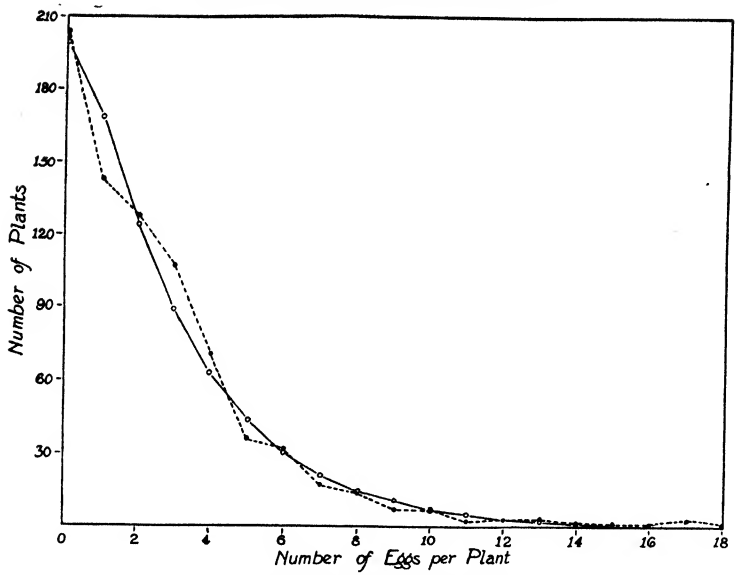


FIG. 5. *First egg count and Greenwood–Yule distribution;* ●----● *= data,* ○——○ *= Greenwood–Yule distribution.*

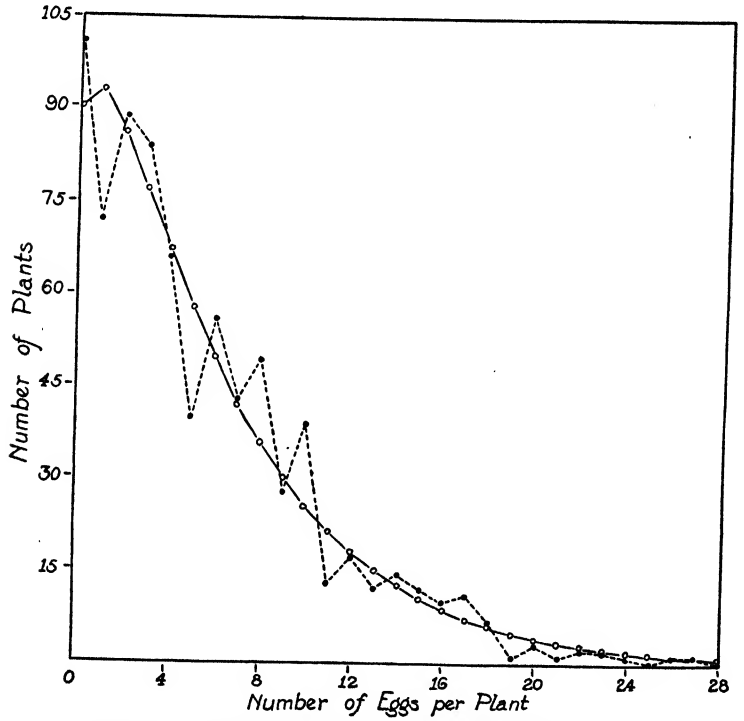


FIG. 6. *Second egg count and Greenwood–Yule distribution;* ●----● *= data,* ○——○ *= Greenwood–Yule distribution.*

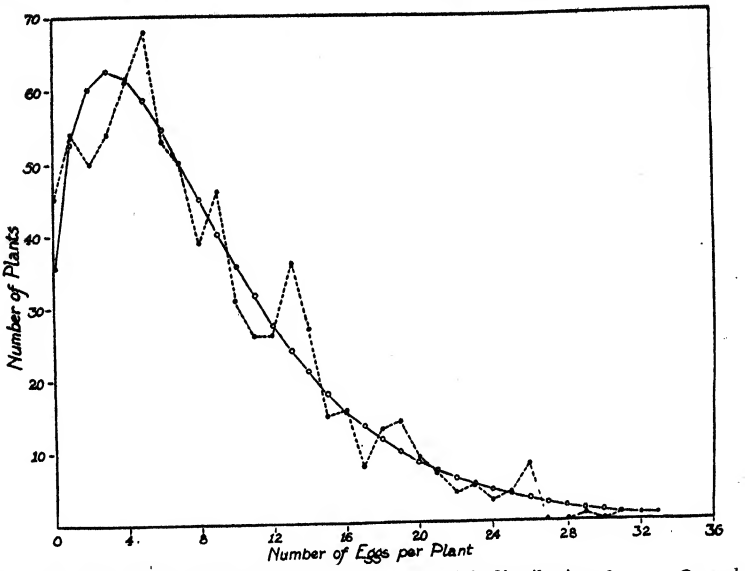


FIG. 7. *Sum of the two egg counts and Greenwood–Yule distribution;* ●- - - -● = *data,* ○———○ = *Greenwood–Yule distribution.*

from the data with which it is to be compared, the curve tends to adjust itself to the needs of the data. On the other hand, with the author's method of compounding an arbitrary series of simple random distributions into a single curve, it was desirable to keep the number of these distributions as low as possible in order to avoid too much clumsy arithmetic. The very attractive plants are therefore treated as one group, and although the resulting curve is asymptotic to the horizontal axis, the ordinate value tends too rapidly to zero.

The Greenwood–Yule frequency for the sum of the two counts fits the data fairly well for the numbers of eggs greater than five per plant, but it reaches a maximum in the wrong place. In this respect it is, however, better than the theoretical distribution of Fig. 3. At this stage, to recall the difference between the construction of Fig. 3 and Fig. 4, may help to explain what seems to be the inadequacy of the Greenwood–Yule curve in this particular case. In Fig. 3 the plants are assumed to have retained the same *relative* attractiveness during the oviposition periods prior to each count. In Fig. 4 the plants most attractive in the first count are assumed at the second count to be on the decline in respect of their choice by moths, and to have been superseded by others. The Greenwood–Yule distribution for both counts together was derived directly from the actual distribution for the sum. The constants about which it is built up are therefore those most suited to illustrate the data. What it cannot allow for is the essential difference between the sum and each of its two component parts. Within each count there is a continuity of events, but between the counts there is a distinct gap. The interval between the two occasions of counting the eggs was a week and it is recorded that

Heliothis eggs hatch in three or four days. Therefore there was no record of the eggs laid during the three days succeeding the first count. If the author's hypotheses are correct, their distribution would presumably have indicated an intermediate stage in the relative attractiveness of the maize plants.

The important point is that the Greenwood–Yule distribution assumes that there is variation among the plants in the likelihood of their receiving each of the individual eggs. It was mentioned above that the original problem to be investigated was the occurrence of accidents among factory workers, the hypothesis being that the persons concerned varied in their tendency to meet with accidents. For accidents to happen the passage of time is required, particularly if some individuals are to be involved in more than one of the accidents. But the individuals themselves are not considered to be affected by the passing of time. Those who at the outset are not likely to meet with accidents remain like that. Those who are prone to accidents do not become more careful as the result of mishaps. The susceptibility of each person is determined by his inherent physical and mental make up. Most people know from experience that there is a certain amount of truth in the saying that a burnt child dreads the fire, but a general theory based on a parallel idea did not explain the distribution of accidents (1). The mathematical formula that did in fact give adequate representations of the accident distributions incorporated the theory that the individual persons differed in their liability to accidents, but did not learn through experiencing them to avoid them. This same formula does, as we have shown, give a fairly good representation of the distribution of *Heliothis* eggs among the plants in a plot of maize. It is reasonable to suppose that the plants vary in their attractiveness to the female moths; i.e., in their liability to receiving eggs. So far, the basic hypothesis of the formula is acceptable, but it is not quite adequate to explain all the facts in the present problem.

Discussion

The object of this paper is to explain the observed distribution of the eggs of the moth, *Heliothis armigera*, on maize, which is one of the insect's most important host plants.

The observed distribution, when plotted by graphical methods, presents certain irregularities in its details, but its general trend can be represented by a smooth curve, the appearance of which suggests that the phenomena that it represents conform to some mathematical law.

It is possible that the irregularities themselves are significant and due to errors in sampling of some particular kind; but this suggestion is derived from cases that have little or no relation to those discussed in the paper and the known facts do not support it.

The observed distribution of the eggs of *Heliothis* on the plants represents the end result of a multitude of complex and continuous interactions between the insect, the host plant, and the rest of the environment. These interactions cannot be brought under direct observation and it is impossible to

retrace the history of the events in the fields studied. We cannot, therefore, subject our explanation to an exact observational test.

Nevertheless, there are two criteria by which we can judge its value. We are dealing, in the first place, with a series of numbers, arranged in a certain manner. We have formed the impression that it is an *ordered series*; i.e., one in which the values follow one another according to a *mathematical law*. When we have discovered the mathematical law, we have one of the most important parts of the explanation. In the second place, we have to deal with actual living organisms of certain specific kinds, behaving in certain characteristic ways that have been observed and described. Our explanation must take these into account, or, at least, it must agree with such observed facts as are relevant to the process that we are discussing.

It is clear that *both* requirements must be met if our explanation is to be considered adequate. To find the mathematical law of the distribution (supposing, which may not be the case, that its law can be defined with absolute certainty) is not enough. We could not *explain* the form of a liquid drop completely by proving that it had the equation of a sphere; we would have to show, in addition, how the known properties of liquid matter *actually engendered this form*. Similarly, in the present case, we must not only find the mathematical law according to which the eggs of *Heliothis* are distributed; we must also show the connection between this law and the observed characteristics of the moth and the developing maize plant. An explanation that is based on physical and biological phenomena of other categories and that has no demonstrable connection with moths and maize plants, cannot be accepted as adequate, even though its mathematical results agree well enough with the data.

We can go even farther than this. Because, owing to inevitable errors in sampling and to the irregularities in natural phenomena, trends that are truly fundamental may be distorted and masked, it is usually difficult to be quite sure just what mathematical law a given process really follows. The tendency to choose a curve that fits reasonably well simply because it is derived from a known mathematical expression, may lead us badly astray. It may be, that the true mathematical law, based on the biological facts, is less elegant or easily expressed and will not reveal itself unless the facts are taken as a starting point. In short, our explanation must be a *biologico-mathematical explanation* and since we are in the domain of natural history, we are justified in insisting on the outstanding importance of its biological element.

In the region where the data were collected *Heliothis* breeds continuously for a considerable period, and, owing to the overlapping of the generations there is, during a good part of this time, a large floating population of moths ready to oviposit.

Parsons and Ullyett proved definitely that the attraction of the plants to the moths is a quality that changes with the passage of time. They demonstrated (1) that the appearance of moth eggs in a particular crop of maize is due to the attraction of moths from neighbouring regions, and (2) that when the

oviposition rate diminishes, it means that the surviving moths have left for other, more attractive areas. Thus, the rate of oviposition at any time depends on the number of moths present, that is to say, on the degree of attractiveness of the crop as compared with other neighbouring crops.

As the plants in a field begin to mature, the moths in the neighbourhood are attracted in increasing numbers to the field. The rate of oviposition rises rapidly and the number of eggs attains a maximum by the time all the male flowers or tassels have appeared. At this time only 42% of the tassels are fully extended and only 50% of the silks have appeared, but 70% of the eggs have been laid. When, later, all the tassels are completely developed, the rate of oviposition is almost negligible and by the time that 100% of the cobs have full silking, oviposition has ceased in that field.

Marshall's data showed that the eggs are certainly not distributed either uniformly or at random among the plants. The obvious inference is, that some plants are more likely to receive eggs than others. We may take it that the movements of the moths are random movements in the sense that they do not search the plot of maize in an orderly or systematic manner: do not, for example, go up one row of plants and down the next. We may also call them random, meaning that the movement of a given moth from its resting place may be to *any* point of the compass. But this is merely because the most attractive plant at the moment may be at any point of the compass, since there is no definite relation between the spatial arrangement of the plants and the degree of attractiveness. Though the movements of the moths would seem random to a casual observer and may really be random, with respect to compass points, they are not random with respect to the character of the individual host plants. However, since the attractiveness of the whole crop changes, increasing to a maximum and then diminishing, according to the proportion of plants that are in the various stages of development, it is not unreasonable to assume that with the passing of time there is a change in the relative as well as in the absolute attractiveness of the individual plants. Surveys made at different times could not, therefore, be expected to give similar spatial distributions of eggs.

Now the type of explanation of egg distribution that we are attempting to produce requires the description of the biological situation in a form susceptible of mathematical representation. Only quantitative elements can be included in the mathematical representation. Nevertheless, the pattern or arrangement of these elements must be that of the real situation: in this case, a pattern characteristic of the moth population ovipositing in the maize fields. But one can imagine a series of such patterns all based on events in the maize field, but differing greatly in complexity. Thus the problem of the oviposition of *Heliothis* could be called the problem of distributing x units among y units. It would then be mathematically simple, but would probably not lead to a correct solution because certain important features of the real problem had been omitted. On the other hand, an effort to include everything that really affects the distribution of *Heliothis* eggs might result in a mathematical repre-

sentation so complex as to be unworkable. Only a biologist acquainted with natural conditions and the behaviour of the organisms considered can say how many elements must be incorporated in the mathematical outline and even the biologist's judgment is subject to revision as the knowledge of the problem progresses and the results of preliminary attempts at explanation become available.

Let us now review briefly the main possible explanations of egg distribution.

A glance at the data shows that the eggs are not distributed uniformly among the maize plants. For example, all plants do not bear eggs; the plants bearing these have not always the same number nor do they recur in the same places in the plot. The simple idea that the problem is that of x things distributed at random among y things (simple Poisson distribution) was originally tested by Marshall, but as has been already said, the curve developed from this theory differs markedly from the curve of the data. One explanation of this might be that the maize plants differ in their receptiveness to eggs, or, to put the thing in another way, in their attractiveness to the moths. If the maize plot is large enough, and the conditions in it sufficiently varied, there might be so many intermediates between the least and the most attractive plants, at any given moment, that the variation would be practically continuous. The compound Poisson series, derived by Greenwood and Yule, may be applied to the *Heliothis* problem if we assume that the variation of the maize plants in attractiveness, at any given moment, is continuous and expressed by a skew curve—Pearson, Type III.

There are, however, a number of serious objections to this solution of the problem. In the first place it was developed from the study of an entirely different problem—the distribution of industrial accidents—which has, biologically, nothing whatever in common with the distribution of *Heliothis* eggs in maize plants, although it is possible to construct a vague general definition covering both cases. In the second place, it is not certain that in the relatively small plot studied by Marshall, the maize plants at any given moment formed a continuous series with respect to their attractiveness. It is possible, and even probable that they formed a number of fairly distinct groups, differing rather markedly in attractiveness and connected by relatively few intermediates, because there is often, in a maize crop, a rather marked difference between the dominant and subdominant plants owing to the shade cast by the former. If a graph of the various types were plotted one might, by joining the ordinates, get a rough approximation to the skew curve; but this would not prove that the variation was continuous. In the third place, though the characteristics of the maize plant probably do not form a continuous series in *space*, as is implied in the Greenwood–Yule system, they do almost certainly change in a perfectly continuous manner in *time*, because they wax and wane with the growth of the plant, which is a practically continuous phenomenon. This continuity in time is not taken into account in the Greenwood–Yule system. The system involves some rather difficult mathematics. The constants that define the curve are, as we have seen, derived

from the data so that it is the closest fit that could be obtained for a curve of this class. Nevertheless, it is, in fact, only a moderate fit for the data, so that the defects in its biological foundations are not compensated by the perfection of the numerical results.

The hypothesis developed by the present writer has been erected on the basis of what we actually know about the relation of *Heliothis* during the period of oviposition to the developing maize plant. The object of the work has been, in the first place, to produce this curve from a statement that is biologically accurate. It is not claimed, of course, that the hypothesis is immune to criticism. The representation of the variation in time in the maize plant as a discontinuous process is admittedly diagrammatic and the variation in space at a given moment may be much less definite and discontinuous than is implied in the mathematical treatment adopted. Relatively simple mathematics have been used, because the differences among the maize plants have been considered to be discontinuous. The curves actually obtained, as we have seen, fit the data somewhat better than the Greenwood–Yule curves; and in so far as the combined count is concerned, this is apparently due in part to the fact that the basic Greenwood–Yule hypothesis does not take the facts into account.

It seems therefore justifiable to say that the hypothesis of egg distribution put forward in this paper is satisfactory as a first approximation. In spite of the simplicity of the mathematical treatment employed, the results obtained fit the data rather better than those given by the Greenwood–Yule hypothesis. Further investigations, based on more extensive collections of field data, may enable us to construct a better theory; but no theory, no matter how elegant its mathematical development, nor how exact the numerical 'fit' it gives, can be considered acceptable to biologists unless it is constructed on a foundation of known biological facts.

Acknowledgments

The writer is indebted to the Committee of the Carnegie Trust for the Universities of Scotland for the Fellowship during the tenure of which this investigation was made. Thanks are due also to Dr. W. R. Thompson, F.R.S., for his interest in the problem, and to Mr. G. Udny Yule, C.B.E., F.R.S., for very helpful advice.

References

1. GREENWOOD, M. and YULE, G. U. An inquiry into the nature of frequency distributions representative of multiple happenings with particular reference to the occurrence of multiple attacks of disease or of repeated accidents. J. Roy. Stat. Soc. 83(2) : 255-279. 1920.
2. MARSHALL, J. The distribution and sampling of insect populations in the field with special reference to the American bollworm, *Heliothis obsoleta* Fabr. Ann. Applied Biol. 23(1) : 133-152. 1936.
3. PARSONS, F. S. and ULLYETT, G. C. Investigations on the control of the American and red bollworms of cotton in South Africa. Bull. Entomol. Research, 25(3) : 349-381. 1934.
4. YULE, G. U. On reading a scale. J. Roy. Stat. Soc. 90(3) : 570-587. 1927.
5. YULE, G. U. and KENDALL, M. G. An introduction to the theory of statistics. 11th ed. Charles Griffin and Company, Ltd., London. 1937.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. SEPTEMBER, 1942 NUMBER 9

SMOKED MEATS

I. BACTERIOLOGICAL, CHEMICAL, AND PHYSICAL MEASUREMENTS ON SMOKED AND UNSMOKED BACON[1]

BY W. HAROLD WHITE[2], N. E. GIBBONS[3], A. H. WOODCOCK[4], AND W. H. COOK[5]

Abstract

Wiltshire bacon, cured in pickles of various compositions, was smoked after various maturation periods, and compared with corresponding unsmoked samples during subsequent storage at −9.4° C. Curing practices designed to obtain milder cures were only partly successful, but in all instances it appears that smoking improves the keeping quality.

Smoking reduced the number of surface bacteria to approximately 10^{-4} times the number present before smoking, and effectively retarded growth during storage. The fat of the smoked bacon was much more resistant to the development of rancidity than that of unsmoked bacon. The colour of smoked bacon was significantly lighter than the unsmoked material, and tended to darken more rapidly during storage. Flavour was superior in the smoked samples. From the reduction in bacterial numbers, rate of bacterial growth, and rate of rancidity development, it would appear on the average that smoked bacon would keep about twice as long as unsmoked bacon under comparable conditions.

Introduction

Previous investigations on Wiltshire bacon conducted in these laboratories (2, 3, 4, 6, 11, 12, 13) have been concerned primarily with the effect of certain precuring and curing practices on the quality of the finished product. With the exception of the temperature conditions during transport, less attention has been given to such postcuring treatments as smoking. Recent studies (12, 13) have shown that in ordinary times the British consumer would prefer bacon containing less salt than that shipped from Canada prior to the war. This could only be accomplished by the development of alternative methods for preserving the product during transport. During war there is an urgent need to produce a less perishable bacon in order to avoid losses through delays and less favourable shipping conditions generally. While several supplementary methods of preservation merit examination, many of them appear to be either inadequate or undesirable. Available evidence indicated that

[1] *Manuscript received June 25, 1942.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as paper No. 78 of the Canadian Committee on Food Preservation and as N.R.C. No. 1073.

[2] *Biochemist, Food Investigations.*

[3] *Bacteriologist, Food Investigations.*

[4] *Biophysicist, Food Investigations.*

[5] *Director, Division of Applied Biology.*

smoking might improve keeping quality without introducing objectionable practices or materials. Accordingly investigations on smoked meats were initiated to obtain information on its perishability and quality as compared with unsmoked material.

Observations made in these laboratories during earlier investigations confirmed the general opinion of commercial operators that smoked bacon is, in several respects, less perishable than the unsmoked product. Otherwise there is little definite information, although it has been shown that smoking reduces the susceptibility of the fat to oxidation (7), and it has been stated that surface bacterial growth is reduced (1). Since deterioration can occur in the lean portion due to microbial action, while bacon fat is extremely prone to the development of rancidity through atmospheric oxidation, it is obvious that an effective preservative must inhibit both of these changes. The work referred to above indicates that smoking meets these requirements. In these experiments subjective estimates of flavour and objective measurements of colour of lean, peroxide oxygen content of fat, and surface bacterial counts, were the criteria used for estimating perishability.

Analytical Methods

The sodium chloride, nitrate, and nitrite in the bacon and curing pickles were determined by methods already described (9). Peroxide oxygen was determined by the methods described by White (10). Reference has also been made (5, 6) to the procedures followed for securing and preparing the curing pickles and surface meat samples for bacteriological examination. Colour measurements were made with the improved colour comparator previously described (14).

The relative flavour quality of the smoked and unsmoked samples was determined by a jury group of 18 members of the staff of the laboratories. The samples were tasted two at a time, according to a randomized incomplete block plan, thus ensuring comparable results on all samples. Each member of the jury group tested a pair of samples during the morning and afternoon of three successive days. The attributes of quality judged were: tenderness, colour, saltiness, juiciness, and flavour of lean, and the flavour and texture of the fat.

Materials and Plan

Previous investigations (3, 15, 16, 17) have shown that considerable variation occurs between Wiltshire bacons made in different establishments. If smoking is to be of general utility as a preservative, it must confer additional keeping quality to all classes of product obtained in commercial manufacture. Since the product of different manufacturers was not readily available for this study, the requisite variability for valid testing was produced by employing different cures. These included 30, 25, and 20% brines, a 25% brine without nitrate, and a strong pump and weak cover pickle combination. Seven hogs were selected for uniformity in size and quality, and the 14 sides treated as

shown in Table I. Sides 1 to 8, 12, and 13 were cured in small tanks while the remainder were cured in two groups by a regular commercial cure. Prior to placing in the tanks all sides were pumped in a uniform manner by an experienced operator.

TABLE I

CONCENTRATION OF CURING SALTS IN THE CURING PICKLES, HAMS, AND BACKS STUDIED

Hog	Side	Side No.	Sodium chloride, %					Sodium nitrate, %					Sodium nitrite, p.p.m.				
			Pickle			Ham	Back	Pickle			Ham	Back	Pickle			Ham	Back
			Pump	Cover	Spent			Pump	Cover	Spent			Pump	Cover	Spent		
1	*R*	1	21.3	20.6	19.3	3.75	2.92	0.28	0.48	0.46	0.035	0.051	370	160	120	14	6
	L	2	25.6	25.9	24.0	3.29	3.99	0.29	0.44	0.42	0.020	0.039	330	160	100	12	6
2	*R*	3	25.5	25.9	24.0	4.06	4.36	0.99	0.44	0.42	0.088	0.067	340	160	100	9	6
	L	4	21.3	20.6	19.3	4.13	3.57	0.84	0.48	0.46	0.102	0.064	350	160	120	12	6
3	*R*	5	21.3	20.6	19.3	3.05	3.71	0.28	0.48	0.46	0.009	0.055	220	160	120	10	9
	L	6	25.5	25.9	24.0	3.99	3.90	0.29	0.44	0.42	0.032	0.045	250	160	100	5	5
4	*R*	7	25.4	25.9	24.0	4.61	5.48	1.07	0.44	0.42	0.104	0.100	240	160	100	6	6
	L	8	21.2	20.6	19.3	3.80	3.03	0.92	0.48	0.46	0.069	0.050	200	160	120	7	8
5	*R*	9	30	30	25	4.05	3.62	*R**	*R*	*R*	0.287	0.185	*R*	*R*	*R*	16	14
	L	10	30	30	25	5.62	7.51	*R*	*R*	*R*	0.512	0.288	*R*	*R*	*R*	33	23
6	*R*	11	30	30	25	3.66	3.34	*R*	*R*	*R*	0.324	0.245	*R*	*R*	*R*	13	13
	L	12	21.8	30.3	29.6	3.91	3.27	0.22	0.45	0.42	0.030	0.021	430	570	520	21	16
7	*R*	13	25.6	25.6	24.8	4.72	4.50	0.00	0.01	0.00	0.003	0.00	910	1710	1650	62	85
	L	14	30	30	25	3.42	4.50	*R*	*R*	*R*	0.247	0.322	*R*	*R*	*R*	16	24

* *The composition of the curing pickles employed regularly in the plant in which these investigations were made cannot be disclosed. The values given for sodium chloride are only approximate.*

On removal from cure the sides were allowed to drain and "mature" for 5 to 6 days at 3.3 to 4.4° C. (38 to 40° F.). They were then cut up and the long rib-in English style back and ham used in these experiments.

Each back was divided into three approximately equal portions which were randomized among the following three treatments:

A. Control, stored in unsmoked condition at −1.1° C. (30° F.)

B. Smoked immediately in a commercial smokehouse at 60° C. (140° F.) for about 15 hr. and subsequently held at −1.1° C. (30° F.)

C. Held for 10 days as in *A* and then smoked and treated as in *B*.

When the Group *C* samples had been smoked, all samples were stored at −9.4° C. (15° F.). Before storage they were wrapped individually in sterile brown paper to prevent surface bacterial contamination, and then placed in closed containers to reduce surface drying.

The above conditions were selected to simulate commercial practice. During transport −1.1° C. (30° F.) is about average under normal conditions, while −9.4° C. (15° F.) is probably the lowest temperature generally available for

the storage of bacon in England. Treatments *B* and *C* formed a basis for comparing the effect of smoking in Canada prior to shipment with smoking immediately on arrival in England.

The hams from the 14 sides were sampled for chemical analysis and the remainder used for some preliminary flavour tests to determine the attributes of flavour quality affected by smoking. Hams 2, 3, 6, 8, 9, 11, 12, and 13 were smoked, immediately after cutting, under the conditions described above. These and the remaining unsmoked hams were stored at −17.7° C. (0° F.) to minimize changes during a five-week period until the flavour tests could be made.

Sampling Procedure

Bacteriological Analyses

Bacteriological analyses were made on the pickles used for curing Sides 1 to 10, but not on those used for curing Sides 11 to 14. Pump pickle samples were taken from the pumping needle, cover pickle samples both before and after its entry into the tank, and spent pickle samples from both the bottom and top of the tank before the sides were removed.

Bacteriological examinations were made on Sides 1 to 10 before and after cure. Three square centimetres of the pleural membrane were removed from the 3rd, 7th, and 11th ribs for these analyses. Subsequent analyses were made on the three portions of the 14 backs after maturation, before and after smoking, before storage at −9.4° C. and after 18, 39, and 60 days' storage. For these examinations 6 sq. cm. of the rib or meat surface were used.

Chemical Analyses

Chemical analyses were made on the pump, cover, and spent pickles used for curing all of the sides. Pump pickle samples were taken from the pumping needle, cover pickle from storage tank prior to transfer to curing vat, and spent pickle after removal from the sides and thorough mixing.

After maturation the chloride, nitrate, nitrite, and moisture contents were determined on an approximately one-inch slice removed from the cut surface of the ham, and on a representative sample of the back made up of half-inch slices taken from the front, centre, and rear.

The peroxide oxygen content of the fat from similar samples was determined for all samples prior to storage at −9.4° C. and after storage for 18, 39, and 60 days.

Physical Measurements

Samples were taken for colour measurements from unsmoked backs after maturation, when placed in storage at −9.4° C. (15° F.) and after storage for 39 and 60 days. The smoked samples were observed for colour prior to and after 60 days' storage at −9.4° C.

Results

Observations Prior to Storage

Although the salt content of hams and backs from sides cured in 20 or 25% sodium chloride pickle was usually lower than for the 30% pickle, the differences were less than expected, and failed to reach the level of statistical significance (Table I). This is presumably due to the much smaller meat to pickle ratio associated with the use of the small experimental curing tanks (approximately 5 lb. of meat to 1 gal. of pickle as compared to the normal ratio of approximately 20 lb. to 1 gal. of pickle). The use of the combination of a strong pump and a weak cover pickle appeared to give a satisfactory introduction of the curing salts. The results of comparative flavour tests made by members of the scientific staff and their families on the ham containing no added nitrate (side No. 13) and one of the regular cure containing nitrate (side No. 11) would suggest that the presence of nitrate is not essential for the development of the typical flavour of ham.

Little bacterial growth occurred in the cover pickles containing 20 and 25% sodium chloride during cure, thus agreeing with the results previously obtained for pickles saturated with sodium chloride (6) (Table II). The counts, as determined on 10% sodium chloride agar, were somewhat lower than normally found in this plant (6).

TABLE II

EFFECT OF THE CONCENTRATION OF SODIUM CHLORIDE IN THE COVER PICKLE ON BACTERIOLOGICAL GROWTH, AS ESTIMATED ON VARIOUS MEDIA

Time of sampling	Log. no. of organisms per ml. of cover pickle[1]		
	Nutrient agar	4% Sodium chloride agar	10% Sodium chloride agar
20% Sodium chloride			
Before cure	5.10	5.31	5.48
At start of cure	5.12	5.45	5.52
At end of cure			
Top of tank	4.98	5.20	5.56
Bottom of tank	5.01	5.26	5.58
25% Sodium chloride			
Before cure	5.21	5.35	5.42
At start of cure	5.25	5.42	5.45
At end of cure			
Top of tank	5.18	5.31	5.48
Bottom of tank	5.13	5.22	5.46

[1] *All plates incubated at 20° C.*

Significant increases in the surface bacterial count of the sides, as determined on 4% salt agar, occurred during cure and maturation of material cured in pickles at all three sodium chloride levels (Table III). However, the differences due to method of curing were insignificant. For sides cured in the 20 or 25% brines the rate of growth during cure was essentially the same

TABLE III

EFFECT OF CONCENTRATION OF SODIUM CHLORIDE IN THE COVER PICKLE ON SURFACE BACTERIOLOGICAL GROWTH ON BACON DURING CURE AND MATURATION

Concentration of sodium chloride in cover pickle, %	Bacterial count as log. no. of organisms per sq. cm.			Mean
	Before cure	After cure	After maturation	
20	3.06	4.44	5.59	4.36
25	2.92	4.36	5.30	4.19
30	3.23	4.65	5.18	4.35
Mean[1]	3.07	4.48	5.36	—

Analysis of variance

Variance attributable to:	Degrees of freedom	Mean square
Between cures	2	0.097
Error	7	0.051
Time	2	14.0**
Time × cure	4	0.079
Error	14	0.082

[1] *Necessary difference, 5% level of significance = 0.28.*
** *Indicates 1% level of significance.*

whether the counts were determined on nutrient agar or on 4 or 10% sodium chloride agar (Fig. 1). However, for sides cured in the regular manner the increase in organisms capable of growth on nutrient agar was less than for the other two cures, but corresponded to that found previously (6). Statistical examination of the data, regardless of the method of cure, showed that before cure the counts obtained on nutrient agar and 4% salt agar were essentially the same and significantly greater than those on 10% salt agar. After cure, however, the average counts on 4 and 10% salt agar were the same and significantly greater than those on nutrient agar. It was also found that, while

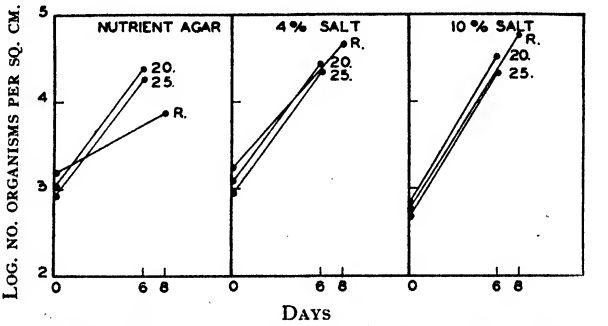


FIG. 1. *Surface bacterial growth on bacon during cure in pickles containing 20, 25, and 30% (R) sodium chloride as shown by counts on nutrient agar and 4 and 10% salt agar.*

significant differences existed between sides before cure, such differences were negligible after cure.

Bacterial Changes During Storage

The effect of the various factors studied on surface bacterial growth during storage are shown by the results given in Table IV. Smoking reduced the number of bacteria on the surface to approximately 10^{-4} times the original number. On samples smoked after maturation a mean logarithmic count of 5.4 was reduced to 1.5, and on samples smoked after storage at −1.1° C. the reduction was from 5.6 to 1.9. Smoking was also effective in retarding surface bacterial growth during storage (Fig. 2). It is of interest to note that at 71 days after maturation there were fewer bacteria on the smoked sides than there were on these prior to smoking. Although sides stored at −1.1° C. for 10 days prior to smoking had a slightly higher mean count than for those smoked after the maturation period, the difference was not significant.

TABLE IV

EFFECT OF METHOD OF CURE AND SUBSEQUENT TREATMENT ON SURFACE BACTERIAL GROWTH ON BACON

Factor	Mean bacterial count[2] as log. no. organisms per sq. cm.
Treatment prior to storage at −9.4° C.	
Unsmoked	7.37
Smoked	2.18
Stored and subsequently smoked	2.55
Time in storage at −9.4° C.	
0 days	3.04
18 days	4.06
39 days	5.00
Method of curing[1]	
20% sodium chloride	4.67
25% sodium chloride	4.31
As for side No. 12	3.02
As for side No. 13	3.34
Regular	3.55

Analysis of variance

Variance attributable to:	D.f.	Mean sq.
Treatment prior to storage	2	352**
Method of curing	4	9.92**
Cure × treatment	8	1.58
Error	27	1.89
Time	2	40.2**
Time × treatment	4	2.78*
Time × cure	8	1.68
Error	54	0.925

[1] *Details of cures as given in Table I.*
[2] *Mean bacterial count for all other conditions over the whole experiment.*
* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

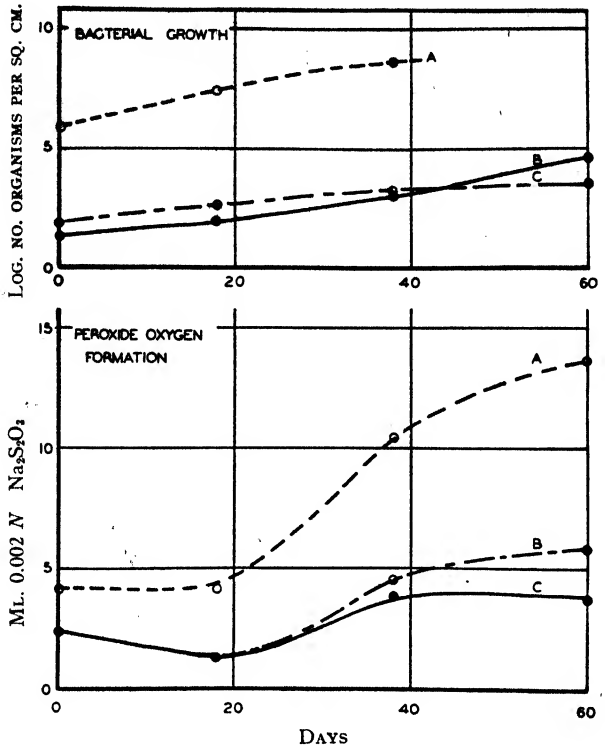


FIG. 2. *Effect of smoking on surface bacterial growth and development of rancidity during storage at −9.4° C. A: unsmoked; B: smoked; C: stored at −1.1° C. for 10 days prior to smoking.*

The mean bacterial counts on sides cured in 20 or 25% brine were significantly greater than for sides cured in 30% brine. However, examination of the detailed results showed that after a storage period of 39 days at −9.4° C. sides cured in the regular manner (30% brine), but unsmoked, had a mean logarithmic count of 8.03 while that for the smoked, mild-cured sides (20 and 25% brine) was 3.77. The use of milder cures is thus feasible if accompanied by smoking prior to shipment or storage.

An analysis of variance of the above data showed that the observed effects of treatment prior to storage, method of curing, and time in storage were all statistically significant (Table IV).

Visual examinations of the product at the time of sampling confirmed the above quantitative results. Unsmoked bacon became slimy and in some instances definitely spoiled after 18 to 39 days' storage at −9.4° C., while that smoked was in good condition with respect to both the fat and lean, even after 60 days, with the exception of one sample upon which a very slight mould growth had occurred.

Development of Rancidity During Storage

The effect of method of curing, period in storage, and smoking on the peroxide oxygen content of the fat is shown by the results given in Fig. 2 and Table V. The values reported in the table are averages over the entire experiment.

TABLE V

EFFECT OF METHOD OF CURE AND SUBSEQUENT TREATMENT ON PEROXIDE OXYGEN FORMATION IN THE FAT OF BACK BACON

Factor	Mean peroxide oxygen content[2] as ml. 0.002*N* $Na_2S_2O_3$
Treatment prior to storage at −9.4° C.	
Unsmoked	8.11
Smoked	3.53
Stored and subsequently smoked	2.82
Time in storage at −9.4° C.	
0 days	3.04
18 days	2.23
39 days	6.29
60 days	7.72
Method of curing[1]	
20% sodium chloride	3.48
25% sodium chloride	3.81
As for side No. 12	3.60
As for side No. 13	6.60
Regular	7.03

Analysis of variance

Variance attributable to:	D.f.	Mean sq.
Method of curing	4	212**
Error	9	14.2
Treatment prior to storage at −9.4° C.	2	926**
Treatment × cure	8	178**
Error	27	7.78
Time in storage	3	572**
Time × treatment	6	107**
Time × cure	12	116**
Error	81	8.85
Analytical error	168	0.0374

[1] *Details of cures as given in Table I.*

[2] *Mean peroxide oxygen content for all other conditions over the whole experiment.*

** *Indicates 1% level of significance.*

The marked instability of bacon fat is demonstrated by the peroxide oxygen levels attained by samples from all treatments within the relatively short period of 60 days. Examination of the detailed results (Fig. 2) showed, however, that the fat from the unsmoked samples was definitely rancid after 39-day storage, while the smoked bacon was sound even after 60 days.

Smoking appeared to be about equally effective in retarding rancidity in the Group *B* and *C* samples. From this it appears that holding the unsmoked product for an additional 10 days at −1.1° C. (30° F.) had no detrimental effect. These results show that the development of rancidity, as determined by peroxide oxygen measurements, is retarded markedly by smoking.

The use of curing pickles containing 20 or 25% sodium chloride gave a more stable bacon fat than those containing 30% sodium chloride. Although this may be due to the effect of sodium chloride on the oxidizing enzyme, lipoxidase, present in pork tissue (8), it may be a reflection of the presence of larger

TABLE VI

EFFECT OF METHOD OF CURE AND SUBSEQUENT TREATMENT ON THE COLOUR OF BACK BACON

Factor	Mean scatter[2], %			
	Red	Green	Blue	Total
Treatment prior to storage at −9.4° C.				
Unsmoked	24.5	14.2	10.9	49.6
Smoked	39.3	26.5	21.3	87.1
Stored and subsequently smoked	38.4	25.7	20.8	85.0
Time in storage at −9.4° C.				
0 days	36.2	23.3	19.0	78.5
60 days	31.9	21.0	16.4	69.3
Method of curing[1]				
20% sodium chloride	34.9	23.1	18.3	76.3
25% sodium chloride	34.3	22.5	17.9	74.7
As for side No. 12	34.1	22.0	17.9	74.0
As for side No. 13	35.0	23.0	18.8	76.8
Regular	32.8	20.6	16.6	69.9

Analysis of variance

Variance attributable to:	Red		Green		Blue		Total	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Method of curing	4	11.2	4	22.1	4	11.6	4	146
Error	9	34.7	9	38.5	9	18.9	9	265
Treatment prior to storage	2	1927**	2	1327**	2	966**	2	12,413**
Treatment × cure	8	30.9	8	28.3	8	16.8	8	213
Error	18	23.3	18	18.7	18	12.1	18	156
Time in storage	1	395**	1	114**	1	142**	1	1803**
Time × treatment	2	105**	2	50.1**	2	45.8**	2	12,128**
Time × cure	4	11.0	4	0.59	4	1.60	4	18.8
Error	27	10.3	27	6.94	27	4.41	27	60.4

[1] *Details of cures as given in Table I.*

[2] *Mean percentage scatter in each band for all other conditions over the whole experiment.*

** *Indicates 1% level of significance.*

amounts of lipoxidase in the rebuilt cover pickle used for the regular cured sides as compared to the diluted and adjusted pickles employed in the experimental cures (8).

The relative importance of the factors studied on spoilage in bacon fat was assessed by means of an analysis of variance. Method of curing, whether smoked or not, and time in storage all contributed significantly to the observed variance. The combined effect of treatment and cure, storage period and treatment prior to storage, and time and cure were statistically significant. The peroxide oxygen content of the fat from sides cured commercially showed marked increase with time of storage while that from the 20 and 25% chloride cures increased at a relatively slow rate. Smoking was effective in retarding peroxide oxygen formation regardless of the nature of cure employed. The peroxide oxygen content of the smoked material increased slowly during storage, while the rate of formation in unsmoked material was considerably greater.

Colour Changes During Storage

The mean values for the colour measurements made with the photoelectric colour comparator, together with a statistical analysis of these results, are given in Table VI. Smoking caused an increase in brightness as shown by an increased light scatter in each colour component. The various cures employed were without effect on the amount of light scattered in any colour component.

An analysis of variance showed that treatment (smoked or unsmoked), storage period, and the combined effect of time and treatment were significant for the red, green, blue, and total scatter. The colour of unsmoked bacon appears to be quite stable since it showed no significant change during storage (Table VII). Although smoking caused a lightening of the colour originally, the colour of the smoked product darkened significantly during storage. There was some indication that the colour of samples smoked immediately after maturation was slightly more stable than that of material held an additional 10 days at $-1.1°$ C. prior to smoking, but the differences were not significant.

Flavour

The results of the flavour tests showed that smoked bacon was superior to the unsmoked in all the attributes of quality studied with the exception of the tenderness and juiciness of the lean (Table VIII). The unfavourable criticism of the smoked product with respect to these two criteria may have been due to drying of the meat to an undesirable extent as a result of the smoking procedure followed. It is also of importance to note that, although the bacon was stored for five weeks prior to testing, the manifestations of the smoking process were still evident at the time of testing. However, since only seven hogs were used in these tests, the results are considered as being primarily of value in giving an indication only of the effect of smoking on the various attributes of flavour quality.

TABLE VII

EFFECT OF STORAGE PERIOD ON COLOUR CHANGES OF SMOKED AND UNSMOKED BACON

Storage period, days	Treatment: Unsmoked	Smoked	Stored and subsequently smoked	Mean
		Light scattered, %		
		Red		
0	24.7	41.5	42.5	36.2
60	24.3	37.0	34.4	31.9
Mean	24.5	39.3	38.4	34.1
		Green		
0	14.3	27.2	28.4	23.3
60	14.1	25.8	23.1	21.0
Mean	14.2	26.5	25.7	22.1
		Blue		
0	11.1	22.3	23.5	19.0
60	10.7	20.4	18.1	16.4
Mean	10.9	21.3	20.8	17.7
		Total		
0	50.2	91.0	94.4	78.5
60	49.1	83.2	75.6	69.3
Mean	49.6	87.1	85.0	73.9

TABLE VIII

EFFECT OF SMOKING ON ATTRIBUTES OF FLAVOUR QUALITY IN BACON AS DETERMINED BY JURY GROUP TESTS

Attribute of quality	Type of bacon preferred	Analysis of variance: Variance attributable to: Differences between unsmoked and smoked D.f.	Differences between unsmoked and smoked Mean sq.	Residual D.f.	Residual Mean sq.
Lean					
Colour	*S*	1	5.34*	78	1.29
Saltiness	*S*	1	1.33	78	1.67
Tenderness	*U*	1	2.52	78	1.69
Juiciness	*U*	1	4.69	78	1.70
Flavour	*S*	1	4.08	78	1.69
Over-all preference	*S*	1	0.60	78	8.04
Fat					
Flavour	*S*	1	5.34	78	1.46
Texture	*S*	1	10.1**	78	1.19
All criteria for both fat and lean	*S*	1	7.44	78	10.7

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*
S = *Smoked.*
U = *Unsmoked.*

Acknowledgments

Grateful acknowledgment is made to Messrs. J. W. Ruddell and W. J. Hodder, Canada Packers Ltd., Hull, Que., for their kind co-operation; to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for his advice and aid with the statistical computations; and to Messrs. E. G. Blake and A. J. McLeod, Laboratory Assistants, National Research Laboratories, for their technical assistance.

References

1. CALLOW, E. H. Dept. Sci. Ind. Research (Brit.), Rept. Food Invest. Board for 1931, pp. 135-136. 1932.
2. COOK, W. H., GIBBONS, N. E., WINKLER, C. A., and WHITE, W. H. Can. J. Research, D, 18(4) : 123-134. 1940.
3. COOK, W. H. and WHITE, W. H. Can. J. Research, D, 18(4) : 135-148. 1940.
4. COOK, W. H. and WHITE, W. H. Can. J. Research, D, 18(4) : 159-163. 1940.
5. GIBBONS, N. E. Can. J. Research, D, 18(5) : 202-210. 1940.
6. GIBBONS, N. E. and WHITE, W. H. Can. J. Research, D, 19(2) : 61-74. 1941.
7. LEA, C. H. J. Soc. Chem. Ind. 52 : 57T-63T. 1933.
8. LEA, C. H. J. Soc. Chem. Ind. 56 : 376T-380T. 1937.
9. WHITE, W. H. Can. J. Research, D, 17(6) : 125-136. 1939.
10. WHITE, W. H. Can. J. Research, D, 19(9) : 278-293. 1941.
11. WHITE, W. H. and COOK, W. H. Can. J. Research, D, 18(7) : 249-259. 1940.
12. WHITE, W. H., WINKLER, C. A., and COOK, W. H. Can. J. Research, D, 19(7) : 213-224. 1941.
13. WINKLER, C. A. and COOK, W. H. Can. J. Research, D, 19(6) : 157-176. 1941.
14. WINKLER, C. A., COOK, W. H., and ROOKE, E. A. Can. J. Research, D, 18(12) : 435-441. 1940.
15. WINKLER, C. A. and HOPKINS, J. W. Can. J. Research, D, 18(8) : 300-304. 1940.
16. WINKLER, C. A., HOPKINS, J. W., and ROOKE, E. A. Can. J. Research, D, 18(6) : 225-232. 1940.
17. WINKLER, C. A., HOPKINS, J. W., and THISTLE, M. W. Can. J. Research, D, 18(6) : 217-224. 1940.

FLUORESCENCE AS A MEASUREMENT OF QUALITY IN DRIED WHOLE EGG POWDER[1]

By J. A. Pearce[2] and M. W. Thistle[2]

Abstract

Quality in dried whole egg powder may be objectively assessed by measurement of the fluorescence of a potassium chloride extract of the egg powder after defatting with chloroform. Measurements on a large number of samples of widely varying quality showed that the fluorescence readings were closely correlated with quality ratings assigned by flavour panels. The method attains a satisfactory level of precision, and can be standardized objectively with quinine sulphate.

Introduction

The importance of dried foods is considerably enhanced by war conditions, since drying reduces both the bulk and susceptibility to spoilage of the original food. This is particularly true of egg powders. Although dried egg products were produced commercially prior to the war, most of the material was used for trade purposes. Consequently little attention was given to suitable objective tests for assessing the quality of the superior class of product required for domestic consumption. It appeared desirable under these circumstances to investigate possible objective measurements of quality which offered hope of being at least as sensitive as a subjective flavour test, but without its disadvantages.

The eating quality of a food product is always the final judgment of its desirability. A useful method of quality control, therefore, should be capable of arranging dried egg samples of varying quality in the order of organoleptic choice, and attain a useful degree of reliability and speed.

Considerable deterioration occurs in flavour quality before any change is noted by the chemical tests, such as peroxide oxygen and free fatty acid determinations, usually used on foods of high fat content (unpublished data). Similar behaviour was noted in wheat germ (3). This suggests that changes in the fat are not responsible. Both wheat germ and dried egg powder were found to evolve a sulphur compound when treated with 95% alcohol. An attempt to use this as an indication of quality likewise proved unsatisfactory.

A fluorescence measurement has been used as an indicator of the age of shell eggs (1, 2, 4). However, this measurement was reported to be of no value in determining age unless storage treatment was known (5), suggesting that a change in fluorescence was indicative of deterioration regardless of the cause. The suitability of fluorescence for assessing quality in dried whole egg powders thus appeared to merit investigation.

[1] *Manuscript received July 25, 1942.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 79 of the Canadian Committee on Food Preservation and as N.R.C. No. 1077.

[2] *Biochemist, Food Investigations.*

Methods

Fluorescence

About 5 gm. of egg powder were defatted by hand shaking at room temperature with three 50 ml. portions of fat solvent to give a colourless filtrate through No. 1 Whatman filter paper. The defatted powders were allowed to stand about an hour and a half at room temperature to permit the remaining solvent to evaporate. The defatted powders were extracted by shaking 2.500 gm. with 50 ml. of 10% potassium chloride solution for five minutes. After filtering through No. 1 Whatman paper containing $\frac{1}{4}$ inch of asbestos fibre at the apex, and washing with two 50 ml. portions of potassium chloride solution, the combined filtrate was made up to 250 ml. with 10% potassium chloride solution. The fluorescence of a 15 ml. portion of the extract was determined with the Coleman photofluorometer, using the standard vitamin B_1 filter which transmits light in the region of 365 mμ. The photofluorometer was standardized by adjusting the instrument to give a scale reading of 50.0 for the fluorescence of a solution containing 0.200 γ of quinine sulphate per millilitre.

Data obtained for the calibration of the photofluorometer are given in Table I. The fluorescence reading and concentration of quinine sulphate are related by the equation:

$$y = -0.005346 + 0.003985x$$

where y = concentration of quinine sulphate in γ per millilitre and x = fluorescence reading.

TABLE I

CALIBRATION OF THE PHOTOFLUOROMETER AGAINST QUININE SULPHATE SOLUTIONS OF VARIOUS CONCENTRATIONS

Quinine sulphate γ per ml.	Fluorescence reading	Quinine sulphate γ per ml.	Fluorescence reading
0.40	100.0	0.15	36.2
0.30	81.0	0.10	24.5
0.25	64.5	0.0	4.5
0.20	50.0		

The standard deviation of fluorescence readings on duplicate samples over the entire range of quality was 2.07 units. However, it should be noted that agreement between duplicates was much better on good quality material than on very poor material so that, on the whole, the method can be regarded as possessing a satisfactory degree of precision.

A number of fat solvents were studied in order to select the most suitable for defatting dried whole egg powders. For this purpose fluorescence measurements were made on a series of samples of widely varying history chosen to represent as far as possible the entire range of quality. These samples were extracted with each of the following solvents: chloroform, petrol ether (boiling range, 40° to 50° C.), a mixture of petrol ether and absolute alcohol (3 : 1), absolute alcohol, 95% alcohol, and acetone.

Absolute alcohol and 95% alcohol seemed to remove or destroy a large portion of the fluorescent material during the fat extraction, and were therefore unsatisfactory. Acetone, though paralleling the behaviour of chloroform, was much less convenient to use.

Data for the three remaining solvents are given in Table II, showing correlation with the results of flavour tests obtained by the procedure to be

TABLE II

EFFECT OF SOLVENT ON FLUORESCENCE READINGS AND THE RELATION OF FLUORESCENCE TO ORGANOLEPTIC RATINGS ON EGG POWDERS OF VARYING QUALITY

Solvent	Organoleptic ratings	Fluorescence reading	Correlation coefficient
Petrol ether	8.57	31.0	
	7.65	38.0	
	7.46	36.0	
	6.50	37.0	
	6.43	43.5	
	5.66	34.5	−0.859**
	5.36	33.0	
	5.14	89.5	
	4.90	142.0	
	4.61	98.5	
	0.26	186.0	
3 : 1 Petrol ether–absolute alcohol	8.57	10.5	
	8.00	13.0	
	7.65	10.0	
	7.46	11.0	
	7.00	17.0	
	6.43	11.5	
	6.00	18.0	
	5.66	12.5	−0.845**
	5.14	16.0	
	4.90	15.0	
	4.50	20.0	
	4.11	18.0	
	3.70	18.0	
	3.53	22.0	
	3.13	19.0	
	0.26	45.0	
Chloroform	8.50	27.0	
	8.17	27.3	
	6.42	45.0	
	6.17	53.6	
	5.67	76.1	
	5.66	45.0	
	5.42	66.4	
	3.70	83.0	−0.826**
	3.53	122.2	
	3.13	81.0	
	2.56	82.0	
	1.73	129.5	
	1.53	113.2	
	0.66	142.5	
	0.26	276.0	

** *Surpasses 1% level of significance.*

described. It may be seen that regardless of the solvent used, fluorescence and organoleptic ratings were closely related. Alcohol, even when considerably diluted with petrol ether, reduced the readings appreciably. Chloroform was finally selected as the most suitable solvent, since it gave the greatest range of fluorescence readings between poor and good quality powders.

Palatability

Palatability was assessed by a panel of 14 people, who tasted each sample. The samples were prepared by reconstituting 24 gm. of powder in 100 ml. of distilled water, and heating at 90° C. with constant stirring until the mix reached the consistency of scrambled egg.

Flavour was scored on the basis of a scale used at the Low Temperature Research Station, Cambridge, England†. A score of 10 is allotted for excellent, fresh egg and zero for a repulsive specimen. Eight is the usual level of the best dried egg while six and four are the lowest levels acceptable for use as an egg dish and in baking respectively.

Some work was undertaken to assess the reliability of panel scores and to obtain an indication of the number of persons necessary to secure a reliable judgment. A statistical examination of panel scores (Table III) shows that

TABLE III

ANALYSIS OF VARIANCE OF THE ORGANOLEPTIC RATINGS GIVEN EGG POWDERS BY OTTAWA PANELS

Description	Source of variance	Degrees of freedom	Mean square
Two separate panels on the same material, Saturday	Between panels	1	0.04
	Between duplicate samples unknown to taster concerned	28	0.88
	Between samples	7	44.6**
	Between tasters tasting duplicate groups of four samples	14	7.77**
	Error	77	0.97
Two separate panels on the same material, Monday	Between panels	1	28.0**
	Between duplicate samples unknown to taster concerned	28	1.71
	Between samples	7	32.1**
	Between tasters tasting duplicate groups of four samples	14	6.39**
	Error	77	1.61
On the five samples duplicated Saturday and Monday	Between days	1	1.60
	Between samples	4	55.8**
	Samples × days	4	4.06
	Error	130	3.18

** *Surpasses 1% level of significance.*

† *Private communication.*

duplicate samples are not confused by the same taster, but duplicate groups of samples are scored differently by different tasters. This indicates that comparisons play a large part in a taster's judgment. It may also be seen that, while on one day two separate panels of seven persons agreed on the same material, two days later the panels differed on the same material. However, although the panel differences were wider on the second trial, the same average ratings were given to the five samples tested on both days. These results indicate that seven persons will give a fairly good judgment on this particular scheme, but at least 14 persons are necessary to obtain a sound rating.

Interrelation of Flavour Score and Fluorescence

The suitability of the fluorescence method was assessed by the relationship of the results to those obtained by flavour tests.

The material consisted of two sets of samples; a series with varying history chosen to represent as far as possible the entire range of palatability and quality, and a series consisting of samples of powder collected from the secondary dust collectors of various commercial plants. The latter material was freshly dried but had usually suffered some deterioration.

TABLE IV

FLUORESCENCE READINGS ON EGG POWDERS FROM VARIOUS COMMERCIAL SECONDARY DUST COLLECTORS

Organoleptic rating	Fluorescence reading	Organoleptic rating	Fluorescence reading
8.38	15.0	5.87	48.4
7.87	16.4	5.63	42.5
7.67	28.6	5.36	34.5
7.00	26.0	5.23	39.7
6.93	22.8	5.20	55.5
6.86	36.8	4.82	58.8
6.79	19.0	4.80	55.8
6.47	18.0	4.07	48.0
6.03	33.4	3.77	55.7

Data from Table II, Part 3, and Table IV are combined to give Fig. 1, in which the logarithms of photofluorometer readings are plotted against taster scores. The correlation coefficient was -0.902; i.e., the fluorescence reading increases with decreasing flavour quality, and excellent agreement is indicated between the two. From the equation for this data, $y = 2.32 - 0.12x$, (where y = the logarithm of the photofluorometer reading and x = organoleptic rating), it is evident that 38.9 and 67.6 photofluorometer units correspond to organoleptic ratings of 6 and 4 respectively. Reference to Table I and the equation relating concentration of quinine sulphate with fluorescence shows that these photofluorometer readings correspond to solutions of 0.150 γ and 0.264 γ of quinine sulphate per millilitre.

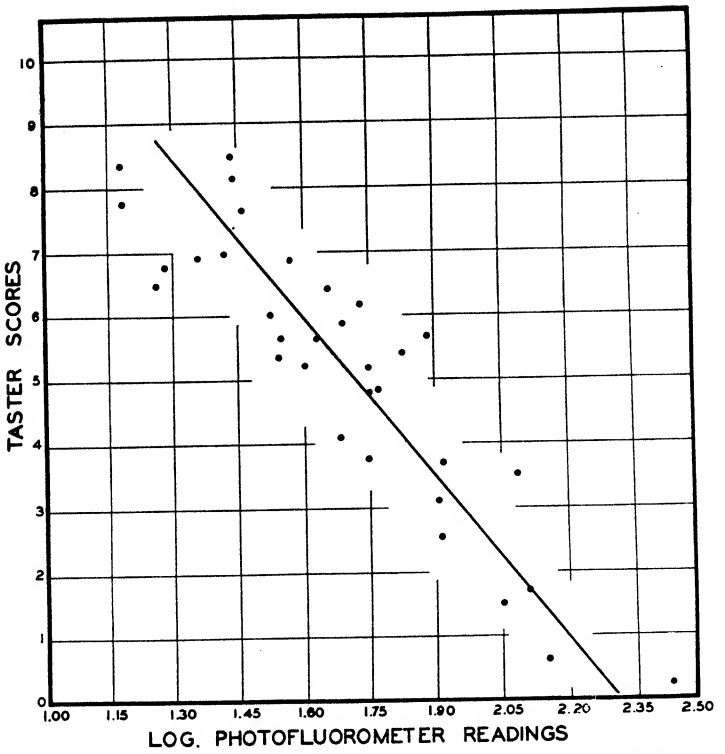


FIG. 1. *Relation between taster scores and the logarithms of the photofluorometer readings.*

An analysis of covariance on the two distinct sets of samples tested indicates a difference between the correlation curves obtained, equivalent to about one unit in palatability score. While the difference is statistically significant, no definite reason can yet be given for this behaviour. Variations in the judgments of the tasters with time, or a variable behaviour of the material responsible for fluorescence may have been responsible. Both of these possibilities are being investigated.

Discussion

The high correlation between fluorescence measurements and palatability indicates that this test should be useful in common practice. It is adaptable to commercial use, can be standardized with objective standards (quinine sulphate solutions), and attains a satisfactory level of precision.

On the samples tested, a high level of correlation has been demonstrated between fluorescence readings and palatability scores. However, it cannot yet be definitely stated that this test will apply to all forms of deterioration that may occur under adverse commercial practice or storage conditions.

In less extensive experiments, this measurement has been found to reveal deterioration that occurs when dried whole egg powders are held at tem-

peratures of 37° C. In addition it is sufficiently sensitive to detect differential deterioration at the outside and centre during cooling of a freshly filled barrel.

One of the most difficult forms of deterioration to assess objectively is that occurring in powders having off-odors. Certain samples with a relatively offensive odour may rate reasonably high in a palatability test on the scrambled egg powder. Presumably, the malodorous substances are volatilized during the cooking process. The results of fluorescence tests on a few special samples in this class, collected from commercial sources, appear in Table V. With the possible exception of the rarely occurring fishy odours, it appears that the fluorescence test is capable of detecting the various types of deterioration represented.

TABLE V

FLUORESCENCE READINGS ON EGG POWDERS FROM COMMERCIAL SOURCES

Classification	Fluorescence reading
Fishy	26.2
Good	29.9
Very fishy	35.1
Gritty	35.2
Badly scorched	65.4
Mottled	65.9
Collector	75.6
Slightly scorched	86.0
Mouldy	157.0

Nothing is yet known about the nature of the changes responsible for the increase in fluorescence, although this is also under investigation.

Acknowledgments

The authors wish to express their thanks to Messrs. R. T. Legault and M. L. McMahon, Laboratory Assistants, for valuable technical aid, and to Mr. F. H. Smith, Statistical Assistant, who made the necessary calculations.

References

1. DINGEMANS, J. J. J. Chem. Weekblad, 28(23) : 350-351. 1931.
2. DINGEMANS, J. J. J. Chem. Weekblad, 29(9) : 138-140. 1932.
3. PEARCE, J. A. In preparation.
4. WAEGENINGH, J. E. H. VAN and HEESTERMAN, J. E. Chem. Weekblad, 24 : 622-623. 1927.
5. ZÄCH, C. Mitt. Lebensmitt. Hyg. 20 : 209-215. 1929.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. OCTOBER, 1942 NUMBER 10

THE HAEMOLYTIC ACTION OF PHENOTHIAZINE DERIVATIVES[1]

BY H. BRUCE COLLIER[2] AND DELLA E. ALLEN[3]

Abstract

The haemolysis of horse erythrocytes by saponin or by lysolecithin, *in vitro*, is powerfully accelerated in the presence of phenothiazone, thiazine S-methyl sulphonium perchlorate, and especially the urinary conjugate, potassium leucophenothiazone sulphate. Bile salt haemolysis is only slightly affected.

Sheep cells are more resistant to haemolysis and to its acceleration by these derivatives.

Phenothiazone does not produce photodynamic haemolysis.

This acceleration of haemolysis is suggested as an explanation of the haemolytic anaemia frequently observed in some species after treatment with phenothiazine.

Introduction

Phenothiazine, when used as an anthelminthic, is relatively non-toxic in sheep (25) and most other laboratory and domestic animals (28). In horses, however, relatively small doses of the drug may cause an acute haemolytic anaemia; the nature of this anaemia has been studied by Swales, Collier, and Allen (27). In humans, also, a haemolytic anaemia frequently results from phenothiazine therapy (13, 17) and one fatality has been recorded (1).

The recent review of Haden (12) indicates that little is known of the mechanism of the anaemias caused by drugs. In the case of the phenothiazine derivatives, tests in this laboratory have failed to reveal any direct haemolytic action upon horse erythrocytes *in vitro*. Finally, however, advantage was taken of the technique of Ponder (19) to demonstrate that some of the soluble derivatives *accelerate* the haemolysis of red cells that is produced by lysins such as saponin and lysolecithin. The hypothesis is advanced that the drug may exert its haemolytic action *in vivo* through this accelerating effect.

Experimental

Materials

The saponin used was the product of Eimer and Amend. Lysolecithin was prepared from egg yolk by the method of King and Dolan (15), using rattlesnake venom* as the source of lecithinase.

[1] *Manuscript received July 8, 1942.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Lecturer. At present, Assistant Professor of Biochemistry, Dalhousie University, Halifax, N.S.*

[3] *Research Assistant.*

* *We are indebted to the Technical Division, Sharp and Dohme, Glenalden, Pa., for a generous supply of dried Crotalus atrox venom.*

Phenothiazone (3-oxyphenothiazine) was prepared by the method of Pummerer and Gassner (21). N-methyl phenothiazine and thiazine S-methyl sulphonium perchlorate were made according to Bernthsen (3) and Kehrmann and Dardel (14) respectively. Conjugate—potassium leucophenothiazone sulphate—was recovered from the urine of treated sheep and horses by the method of Collier (7) and was recrystallized several times from water. Synthetic conjugate was prepared from leucophenothiazone by the method of Burkhardt and Lapworth (6).

Methods

Horse and sheep blood were collected directly into a preservative anti-coagulant fluid and were stored at about 4° C. The fluid first used was the glucose–citrate solution of Denstedt (11), but washed horse cells were found to be somewhat unstable when suspended in it. Finally, Denstedt's (10) modified preservative fluid* was found to be quite suitable. This solution was used throughout for storing, washing, and finally suspending the cells.

One volume of blood was collected into ½ volume of this fluid. The cells, before testing, were washed three times and were made up to a concentration of 1 : 3 by volume. To 10 ml. of the solution was added a suitable small volume of stock lysin solution (saponin or lysolecithin) and 0.10 ml. of the cell suspension, giving a final concentration of red cells of 1 : 300 by volume. In testing a compound for acceleration, it was first dissolved in the fluid, then the lysin and cells were added, in that order.

Haemolysis was allowed to proceed at room temperature, the amount of lysin being sufficient to cause complete haemolysis in about 30 to 60 min. The breakdown of the cells was followed by observing the change in opacity in a Klett-Summerson photoelectric colorimeter equipped with a red filter (Corning No. 241). The reading at 100% haemolysis gave a blank value which was used to calculate percentage haemolysis from the other readings. As seen in Fig. 1 the haemolysis curves are sigmoid; 50% haemolysis was taken as the end-point, because of the difficulty in estimating the exact time for complete lysis.

The time for 50% haemolysis of a standard cell suspension by a known amount of lysin was first determined. Then the time required for the same amount of lysin to cause 50% haemolysis in the presence of the test substance was ascertained. (In no case did the test substance alone, in the absence of lysin, cause haemolysis.) The acceleration was then expressed as the quotient, Q = time with lysin alone ÷ time with lysin plus accelerator.†

* *100 ml. 5% glucose.*
75 ml. 3% sodium citrate.
75 ml. isotonic phosphate buffer, pH 7.4.

† *This factor is roughly the reciprocal of Ponder's "R," which is the ratio of lysin concentrations necessary to give complete haemolysis in the same period of time, i.e., concentration in presence of accelerator ÷ concentration in absence of accelerator.*

As the main purpose of the present paper is to point out the accelerating effect of the phenothiazine derivatives, it was found simpler to employ a fixed lysin concentration and to observe the difference in time interval. It is planned to investigate the kinetics of this acceleration in greater detail, at which time the conventional methods of expression will be employed.

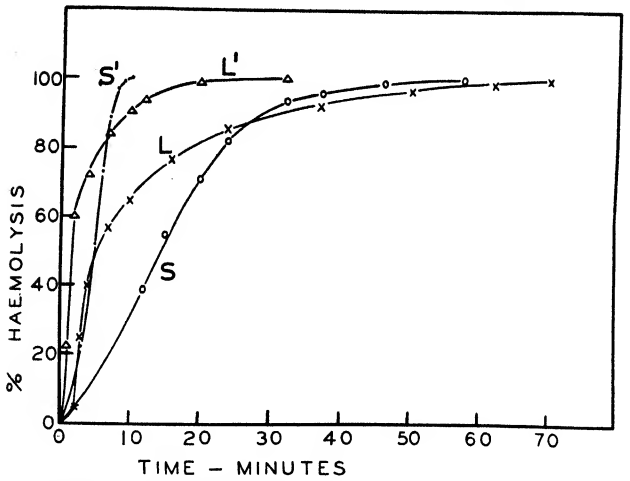


FIG. 1. *Haemolysis of horse erythrocytes. S, saponin alone; S', saponin + 2 × 10⁻⁵ M phenothiazone; L, lysolecithin alone; L', lysolecithin + 3 × 10⁻⁵ M phenothiazone.*

Acceleration of Saponin Haemolysis of Horse Cells

Fig. 1 illustrates the nature of the time–haemolysis curve obtained by the action of saponin on horse erythrocytes. It demonstrates also the accelerating effect of phenothiazone at 2×10^{-5} *M* concentration. Table I gives the relationship between the concentration of phenothiazone and the degree of acceleration observed.

TABLE I

EFFECT OF PHENOTHIAZONE ON SAPONIN HAEMOLYSIS OF HORSE CELLS

Cells, 1 : 300; saponin, 25 μg. per ml.

Phenothiazone concentration, $M \times 10^6$	Time for 50% lysis, min.	Q
0	14	1.0
2.0	13	1.1
7.4	9	1.6
20	5	2.8
35	3	4.6
74	2	7.0

Cells suspended for 30 min. in 3×10^{-5} *M* phenothiazone and then washed were no more fragile to saponin than were untreated cells. (It was noted that some methaemoglobin was formed, indicating penetration of the cells.) Ponder (18) similarly found that cells treated with solutions of benzene and of indole showed no permanent effects if washed immediately.

The conjugate isolated from horse urine was found to exert a very powerful accelerating effect; the same was true of the synthetic conjugate. As it is

impossible to obtain these derivatives in a high degree of purity, the results for the two were not identical, as seen in Table II. Furthermore, the concentration of conjugate was found to be very critical, which made it more difficult to obtain precise Q values.

TABLE II

EFFECT OF CONJUGATE ON SAPONIN HAEMOLYSIS OF HORSE CELLS

Accelerator	Time for 50% lysis, min.	Q
Horse conjugate,		
0	30	1
1.1×10^{-5} M	10	3
5.6×10^{-5} M	< 1	> 30
Synthetic conjugate,		
0	25	1.0
3×10^{-5} M	30	0.8
3×10^{-4} M	11	2.3
3×10^{-3} M	1	25

Other compounds, listed in Table III, were tested, but in this series the effect of varying concentration was not determined.

TABLE III

ACCELERATION OF SAPONIN HAEMOLYSIS OF HORSE CELLS

Substance	Concentration, M	Q
Thiazine S-methyl sulphonium perchlorate	0.0016	10.9
N-methyl phenothiazine	0.0012	1.2
Quinine	0.0005	6.8
Indole	0.0020	2.7
Sulphanilamide	0.010	1.5
Acetylcholine	0.0020	1.0

Haemolysis of Sheep Cells

Sheep erythrocytes are generally believed to be less fragile than horse erythrocytes. With saponin, it was found that sheep cells required about three times the concentration to produce the same rate of lysis as horse cells. Further, the accelerating effect of the phenothiazine derivatives was appreciably less, as seen in Table IV.

Inhibition of Haemolysis by Serum

Blum (4) and Tsai and Lee (29) have demonstrated that there is an antihaemolytic substance in normal serum. Under the conditions herein described it was confirmed that very little serum was required to neutralize the amount

TABLE IV

SAPONIN HAEMOLYSIS OF SHEEP CELLS

Cells, 1 : 300; saponin, 75 μg. per ml.

Accelerator	Time for 50% lysis, min.	Q
None	20	1.0
Phenothiazone, 0.0002 *M*	6	3.3
Sheep conjugate, 0.0003 *M*	12	1.7
Sheep conjugate, 0.003 *M*	4	5.0

of saponin used, and that this antihaemolytic effect was also exerted in the presence of the accelerators. The effect of serum upon saponin lysis of horse cells, in the presence and absence of accelerators, is summarized in Table V.

TABLE V

EFFECT OF SERUM UPON SAPONIN HAEMOLYSIS OF HORSE CELLS

Serum concentration	Time for 50% lysis, min.	
	Saponin alone	Saponin plus *M*/1000 conjugate
0	15	Instantaneous
1 : 250	13	Instantaneous
1 : 100	20	6
1 : 80	> 240	> 240

Lysolecithin Haemolysis of Horse Cells

Since lysolecithin is believed to be a normal lysin in the mammalian circulation, the effect of accelerators on its action was examined. This lysin was not investigated extensively, as it was found difficult to obtain reproducible results. This was shown to be due to the fact that the activity of the lysolecithin decreased rapidly in very dilute solution. (This is presumably the reason why the rate of haemolysis falls off so markedly as complete lysis is approached.) Nevertheless, the results obtained were sufficient to demonstrate that the same typical acceleration of haemolysis occurred in the presence of phenothiazone and of the conjugate.

Fig. 1 illustrates the normal time curve of haemolysis of horse cells by lysolecithin (7 μg. per ml.) and the accelerated curve in the presence of 3×10^{-5} *M* phenothiazone; from the times for 50% lysis, Q is found to be about three. The conjugate had a very powerful effect upon lysolecithin action, Q values of approximately 25 to 50 being obtained. Because of the difficulty in obtaining precise quantitative data, the observations are summarized qualitatively as follows:—

Horse conjugate, 1.3×10^{-5} *M*...no acceleration.
1.3×10^{-4} *M*...instantaneous lysis.
Synthetic conjugate, 4×10^{-6} *M*...slight acceleration.
4×10^{-5} *M*...instantaneous lysis.

Serum inhibited lysolecithin haemolysis, and its acceleration, just as in the case of saponin. Serum at 1 : 50 dilution completely prevented haemolysis by the lysin alone; it inhibited also the haemolysis in the presence of lysin and 0.001 *M* conjugate. Serum at 1 : 500 slightly inhibited both normal lysis and that taking place in the presence of conjugate.

Bile Salt Haemolysis of Horse Cells

Horse erythrocytes were lysed in the presence of 0.026% sodium choleate (Merck). Phenothiazone, however, had only a very slight accelerating effect. In the presence of 5×10^{-4} *M* phenothiazone, the time for 50% haemolysis was reduced from 18 min. to 9 min., giving a *Q* value of 2.0.

The Effect of Light

Blum (5) has demonstrated photodynamic haemolysis in the presence of dyes such as rose bengal. It was thought that the phenothiazine derivatives might show a similar behaviour, especially as a photosensitizing action of phenothiazine has been demonstrated by DeEds (9) in humans, and by Swales *et al.* (26) in pigs.

A standard suspension of horse cells in 10^{-4} *M* phenothiazone was irradiated for one hour at room temperature by the light of a small carbon arc, through a water cell, at a distance of 30 cm. There was no detectable haemolysis, compared with a dark control, even 24 hr. after the irradiation. It was, therefore, concluded that phenothiazine derivatives probably do not exert their haemolytic effect through photodynamic action.

Discussion

Administration of phenothiazine to horses may cause an acute haemolytic anaemia, but the various derivatives of the drug that have been tested *in vitro* have no direct lytic effect on horse erythrocytes. It has been shown, however, that some of these compounds have a striking effect in *accelerating* the lysis of the cells by saponin or by lysolecithin, with a much smaller effect upon bile salt haemolysis. This phenomenon is analogous to that described by Ponder (18, 19) in the case of benzene, indole, nonyl alcohol, etc.

It is of interest to note that phenothiazone and thiazine S-methyl sulphonium perchlorate, which are strong accelerators of haemolysis, are also powerful cholinesterase inhibitors (8).

Of especial interest is the very strong accelerating effect of the conjugate, potassium leucophenothiazone sulphate, which is believed to be the derivative of phenothiazine that occurs in the blood of treated sheep (7) and of horses (27). In the horse, we have observed a concentration of conjugate in the plasma of about 0.01%, or 3×10^{-4} *M*, which is sufficient, *in vitro*, to cause

a very marked acceleration of haemolysis. The conjugation of leucophenothiazone to the sulphate is a detoxifying mechanism; nevertheless it may actually intensify the haemolytic effect.

To apply this accelerating effect of the phenothiazine derivatives, observed *in vitro*, to explain intravascular haemolysis involves several assumptions. First, it may be assumed that circulating erythrocytes are normally being broken down by a lysin, such as lysolecithin, as has been proposed by Bergenhem and Fåhraeus (2). (See, however (24).) It would then be assumed that the phenothiazine derivatives in the blood can markedly accelerate this normal cell breakdown.

Support for this hypothesis may be found in the fact that indole is an accelerator of haemolysis (19), and Rhoads (22) has demonstrated that indole is haemolytic in dogs, especially on a vitamin-B–free diet. Furthermore, the accelerating effect of quinine, described by Ponder and Abels (20) and confirmed by us, has been used by Mer and co-workers (16) to explain the acute haemolytic anaemia frequently observed in malaria patients treated with quinine. These workers showed that quinine could accelerate bile salt haemolysis.

The inhibitory effect of serum on erythrocyte lysis may seem to be an objection to this hypothesis. However, conditions in the circulation are not identical with those *in vitro*, and it is believed that the erythrocyte exists in a state of more or less unstable equilibrium, being constantly subject to lytic tendencies. If so, the presence of accelerating drugs may quite possibly hasten the process of lysis, which presumably is taking place continually in the normal animal.

In order to test the effect of the vitamin B complex on the phenothiazine anaemia, rabbits and guinea pigs were kept on a relatively B-free diet. Large and repeated doses of the drug failed, however, to produce any significant degree of anaemia (Swales, Collier, and Allen, unpublished data.) Schnitzer, Siebenmann, and Bett (23) had a similar experience with white mice and guinea pigs on normal diets; with dogs they found no difficulty in producing a marked anaemia.

In conclusion, it seems clear that a more adequate interpretation of the haemolytic anaemias caused by drugs depends upon a more complete understanding of the properties of the normal erythrocyte and of its destruction in the circulation.

References

1. ANON. Lancet, 242 : 86. 1942.
2. BERGENHEM, B. and FÅHRAEUS, R. Z. ges. exptl. Med. 97 : 555-587. 1936.
3. BERNTHSEN, A. Ann. 230 : 73-211. 1885.
4. BLUM, H. F. J. Cellular Comp. Physiol. 9 (2) : 229-239. 1937.
5. BLUM, H. F., PACE, N., and GARRETT, R. L. J. Cellular Comp. Physiol. 9 (2) : 217-228. 1937.
6. BURKHARDT, G. N. and LAPWORTH, A. J. Chem. Soc. : 684-690. 1926.
7. COLLIER, H. B. Can. J. Research, D, 18 (7) : 272-278. 1940.
8. COLLIER, H. B. and ALLEN, D. E. Can. J. Research, B, 20 (9) : 189-193. 1942.

9. DEEDS, F., WILSON, R. H., and THOMAS, J. O. J. Am. Med. Assoc. 114 (21) : 2095-2097. 1940.
10. DENSTEDT, O. F. Data from a forthcoming publication.
11. DENSTEDT, O. F., OSBORNE, D. E., ROCHE, M. N., and STANSFIELD, H. Can. Med. Assoc. J. 44 (5) : 448-461. 1941.
12. HADEN, R. L. *In* A symposium on the blood and blood-forming organs, pp. 83-104. University of Wisconsin Press, Madison. 1939.
13. HUBBLE, D. Lancet, 241 : 600-601. 1941.
14. KEHRMANN, F. and DARDEL, J. H. Ber. 55 : 2346-2356. 1922.
15. KING, E. J. and DOLAN, M. Biochem. J. 27 : 403-409. 1933.
16. MER, G., BIRNBAUM, D., and KLIGLER, I. J. Trans. Roy. Soc. Trop. Med. Hyg. 34 : 373-378. 1941.
17. MILLER, M. J. and ALLEN, D. Can. Med. Assoc. J. 46 (2) : 111-115. 1942.
18. PONDER, E. J. Exptl. Biol. 16 (1) : 38-48. 1939.
19. PONDER, E. J. Gen. Physiol. 25 (2) : 247-261. 1941.
20. PONDER, E. and ABELS, J. C. Proc. Soc. Exptl. Biol. Med. 34 : 162-165. 1936.
21. PUMMERER, R. and GASSNER, S. Ber. 46 : 2310-2327. 1913.
22. RHOADS, C. P. and MILLER, D. K. J. Exptl. Med. 67 (2) : 273-297. 1938.
23. SCHNITZER, R. J., SIEBENMANN, C., and BETT, H. D. Can. Pub. Health J. 33 : 17-24. 1942.
24. SINGER, K. J. Clin. Investigation, 20 : 153-160. 1941.
25. SWALES, W. E. Can. J. Research, D, 18 (7) : 266-271. 1940.
26. SWALES, W. E., ALBRIGHT, W. D., FRASER, L., and MUIR, G. W. Can. J. Comp. Med. 6 (6) : 169-172. 1942.
27. SWALES, W. E., COLLIER, H. B., and ALLEN, D. Can. J. Research. In press.
28. TAYLOR, E. L. and SANDERSON, K. M. Vet. Record, 52 : 635-647. 1940.
29. TSAI, C. and LEE, J. S. Chinese J. Physiol. 16 (2) : 165-178. 1941.

PRESERVATION OF EGGS

II. SURFACE CONTAMINATION ON EGG-SHELL IN RELATION TO SPOILAGE[1]

By F. T. Rosser[2]

Abstract

The method devised to measure surface contamination on shell eggs was to wash eggs with water in a "Waring blendor" and make bacteriological and mould counts on the wash water. To determine the degree of internal contamination, a small electric hand drill was used to cut holes in the shell in order to remove the egg contents under sterile conditions. The egg meats were mixed in the blendor before plating.

External mould growth, after storage for six weeks, under adverse conditions of both temperature and humidity, did not bear any close relation to the amount of mould contamination initially present on the shell. Both oil dipping and handling stimulated the growth of external moulds.

Internal spoilage, as determined by candling at the end of the storage period, was greatest for eggs having a high initial shell count and least for those with a low initial count. Oiling, or disinfecting with hydrogen peroxide followed by an oil dip, did not reduce spoilage. Storage life was increased by strict observance of recognized sanitary practices.

Introduction

It is generally accepted that egg spoilage is usually caused by organisms which enter through the shell (3). If this is true the early elimination of surface contamination followed by sealing the shell pores should considerably reduce losses in storage. This study was undertaken to determine the relation existing between the numbers of bacteria and moulds on the exterior surface of shell eggs and subsequent spoilage in storage, and to assess the value of oil as a sealing agent against the entrance of micro-organisms into the egg.

Methods

Determinations of the number of moulds and bacteria on outer shell surfaces were made by washing eggs in a "Waring blendor"* and plating the wash water on a suitable medium. An open wire basket was constructed to fit closely to the sides of the blendor container and of such a length that the bottom was suspended just above the propeller blades. Three eggs, placed in the basket, were covered by a measured amount of sterile water and the machine operated at high speed for three minutes. Preliminary investigation had shown that further washing removed a negligible number of organisms and tended to raise unduly the temperature of the wash water. Recognized bacteriological technique was used throughout. Eggs were untouched by hand during the experiment, metal lifters being used to move tray lots, and crucible tongs or loops made from stiff wire to manipulate individual eggs. The glass blendor containers and their plastic tops were sterilized in a water-bath the temperature of which was raised to above 80° C. for 10 min. Samples

[1] *Manuscript received September 2, 1942.*
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 80 of the Canadian Committee on Food Preservation, and as N.R.C. No. 1085.

[2] *Mycologist, Food Investigations.*

* *Manufactured by the Waring Corporation, New York, N.Y.*

of the wash water were plated in duplicate on proteose peptone tryptone agar for bacteria and on potato dextrose agar for moulds. Plates were incubated for seven days at 20° C.

To determine quantitatively internal contamination of eggs it was essential that the contents be removed aseptically. The following procedure was found satisfactory for this purpose. The egg was held in a clamp on a ring stand and both ends were painted with iodine. A circle about one-quarter inch in diameter was cut in the shell at the air cell end with a small electric hand drill and a small hole was bored at the opposite end of the egg, care being taken not to puncture the membranes. The cut surfaces were again treated with iodine, after which the excised portion of the shell and the outer membrane were removed with sterile forceps and the membrane below the small hole at the opposite end of the egg was punctured with a sterile needle. The egg was then inverted over a sterile beaker, the lid of which was removed momentarily, and filtered air under pressure applied to the hole in the small end of the egg. In this way the inner membrane was broken and the contents expelled quickly through the large opening, often without breaking the yolk. The meats from the six eggs in each treatment, except those accidentally cracked, were removed as described, poured into the blendor container and mixed for three minutes. Aliquots (1 cc. and 1/10 cc.) were plated in duplicate for the bacterial count. Methods of examination following storage comprised observation of each egg under a strong light for exterior growths and careful candling before a lamp to detect interior defects. All doubtful eggs were broken open and scrutinized under the light.

Experimental

The storage experiment which was begun during the latter part of February was designed statistically in a 6 × 6 Latin square arrangement of half-case units. Each of the six half-case units contained six trays of eggs, each tray of 30 eggs being representative of one of six treatments. Three dozen eggs were secured for each replication of each treatment, six of which were selected at random for initial determinations and the remainder (30) used for the storage experiment. The treatments were as follows:

A. Market eggs (obtained as required from a local egg grading station)
- (1) Grade A Pullet: untreated.
- (2) Grade A Pullet: given oil dip.
- (3) Grade A Pullet: immersed in 3% commercial hydrogen peroxide, dried, and given oil dip.
- (4) Grade A Pullet: immersed in bath containing average of 75,000,000 bacteria and 600,000 moulds per cc.

B. Producer eggs (from a flock kept under excellent conditions)
- (5) Grade A Medium, day-old: graded and handled by producer in usual manner.
- (6) Ungraded, day-old: collected and candled with dry gloved hands.

A commercial, colourless, odourless, tasteless, paraffin base, egg-dipping oil, having a pour point of 40° F. was used for the oil dipping treatments. Each half-case unit was stored for a total period of six weeks under the conditions, indicated in Table I, which were considered suitable for accelerating egg spoilage.

TABLE I

STORAGE CONDITIONS EMPLOYED FOR HALF-CASE UNITS OF EGGS

Half-case No.	Time stored (days)	
	Alternating every two days: 17.6° C. and 95.7% R.H.* 23.5° C. and 69.6% R.H.	Constant: 21° C. and 90% R.H.
1	32	10
2	29	13
3	28	14
4	27	15
5	26	16
6	25	17

*R.H. = *relative humidity.*

The six eggs selected for initial examination were washed in two batches of three each and the average number of organisms was calculated on a "per egg" basis for each treatment. After the eggs had dried they were stored at 0° C. until it was convenient to examine the contents (in no instance for longer than 11 days).

TABLE II

INITIAL BACTERIA AND MOULD COUNTS ON SHELL EGG SURFACES

Condition before washing	Number of eggs washed	Average count per egg	
		Bacteria	Moulds
A. Market eggs			
Untreated*	72	70,000	1600
Dipped in hydrogen peroxide**	36	730	0
Dipped in contaminant**	36	2,780,000	29,000
B. Producer eggs			
Ordinary handling	36	103,000	43
Handled with gloved hands	36	9500	45

* *Includes eggs for control and oil dip treatments.*
** *Allowed to dry off before washing (one to two hours in laboratory).*

Table II shows the results obtained for exterior contamination on the variously treated shell eggs prior to storage. When the difference in size of the eggs was considered, the bacterial count on Grade A market eggs of unknown history was of the same order as that from producer eggs ordinarily handled, but the mould count was about 30 times as high. The producer

eggs untouched by hand, however, showed approximately one-tenth the bacterial count of those handled whereas the mould counts were almost identical. Following hydrogen peroxide treatment counts for both moulds and bacteria were very low, and after dipping in the contaminated bath, very high.

Examination of egg meats revealed the presence of bacteria in only one group of six market eggs (100 bacteria per cc.). This contamination may, of course, have been contributed by only one of the eggs. No moulds were found in any of the meat samples.

The percentages of eggs having external mould and internal mould or bacterial growths at the end of the storage period are given in Tables III and IV, respectively. Because of the differences in percentage of the various amounts

TABLE III

MOULD GROWTH ON EGG SHELL SURFACES AFTER SIX WEEKS' STORAGE

Treatment	Percentage with mould growth on shell						
	Half-case number						Average
	1	2	3	4	5	6	
A. Market eggs							
Untreated	63.3	40.0	60.0	100.0	60.0	80.0	67.2
Oil dipped	100.0	100.0	100.0	100.0	100.0	100.0	100.0*
Hydrogen peroxide treated and oil dipped	96.7	100.0	100.0	100.0	100.0	100.0	99.4*
Dipped in contaminant	66.7	100.0	100.0	90.0	0.0	70.0	71.1
B. Producer eggs							
Ordinary handling	100.0	100.0	16.7	96.7	100.0	30.0	73.9
Handled with gloved hands	6.7	26.7	36.7	10.0	20.0	90.0	31.7

* *Differed significantly from market eggs, untreated.*

TABLE IV

INTERNAL EGG SPOILAGE AFTER SIX WEEKS' STORAGE

Treatment	Percentage spoilage						
	Half-case number						Average
	1	2	3	4	5	6	
A. Market eggs							
Untreated	10.0	13.3	50.0	63.3	80.0	96.7	52.2
Oil dipped	56.7	40.0	86.7	80.0	86.7	73.3	70.6
Hydrogen peroxide treated and oil dipped	30.0	23.3	43.3	76.7	86.7	93.3	58.9
Dipped in contaminant	40.0	76.7	86.7	86.7	70.0	90.0	75.0*
B. Producer eggs							
Ordinary handling	0.0	6.7	33.3	13.3	83.3	63.3	33.3*
Handled with gloved hands	0.0	13.3	40.0	36.7	30.0	60.0	30.0*
Half-case average	22.8	28.9	56.7	59.4	72.8	79.4	

* *Differed significantly from market untreated.*

TABLE V

ANALYSIS OF VARIANCE OF EGG SPOILAGE

Source of variance	Degrees of freedom	Mean square	
		External mould	Internal spoilage
Half-case replicates	5	194.98	1558.46**
Tray position in case	5	743.77	102.62
Treatments	5	2679.99**	1013.31**
Residual error	20	465.38	117.64

** *Exceeds mean square error, 1% level of significance.*

of spoilage the data were transformed to angular measure according to the formula $p = \sin^2 \theta$ (1, Table 12) in order to stabilize the variance for statistical analysis. Table V shows the analysis of variance of these transformed quantities. The effect of initial treatment reached the level of statistical significance for both external mould growth and internal spoilage. In addition significant differences in internal spoilage were observed between replicate half cases. Tray positions within the case had little effect. A statistical analysis of the relation of position in the tray to both external and internal spoilage showed no significant difference in either respect between eggs at the outside of the trays and those on the inside.

The amount of external mould on unhandled producer eggs was less than on those subjected to all other treatments (Table III). Individually these differences were statistically significant for all except untreated market eggs in which the difference almost reached significance. Oil dipped eggs and those treated with hydrogen peroxide followed by an oil dip had more external mould growth than those of any of the other treatments and the differences were statistically significant except between the hydrogen peroxided oil dipped eggs and producer eggs handled in the ordinary manner. Untreated and contaminated market eggs and ordinarily handled producer eggs showed only small differences among themselves in the average amount of external mould growth.

The average percentage of internal spoilage at the end of the storage period was markedly less in producer eggs than in any of the four categories of market eggs (Table IV), and these differences were statistically significant. Although unhandled eggs had the least internal spoilage the difference between the producer egg treatments was very slight. In the market eggs the greatest average spoilage occurred in the contaminated lots and statistical analysis showed the difference between this and the untreated to be significant. While the observed spoilage in the oiled eggs was somewhat greater than in the untreated, the difference was not statistically significant. A progressive increase occurred in the half-case average percentage internal spoilage which was statistically highly significant.

Discussion

There seems to have been no clear cut relation between the number of moulds originally present on the shells of the eggs and their subsequent growth on the surface during storage. Under the severe storage conditions of this experiment, however, it is evident that oil dipping and handling both favoured the growth of exterior moulds. That mould growth is greater on oiled than on unoiled eggs after storage at temperatures and humidities approximating the severest summer conditions, is in agreement with previous work (5).

Since only one of the original 36 groups of eggs showed infection in the egg meats, it seems reasonable to conclude that most of the organisms causing interior spoilage at the end of the storage period entered from the outside. The significant differences found between the spoilage of producer unhandled (low count) and market untreated (medium count) eggs and between untreated and contaminated (high count) market eggs suggest that the amount of shell contamination may play an important part in internal egg spoilage, but since treating market eggs with hydrogen peroxide (very low count) before oil dipping made little difference to either exterior mould growth or interior spoilage, it seems possible that shell penetration by micro-organisms in this instance had proceeded to the point where surface disinfection had little value. On the other hand in spite of the fact that producer ordinarily handled eggs had heavy exterior mould growths, the amount of internal spoilage was small which indicates that fresh egg might be bacteriostatic and mouldistatic.

The progressive increase in internal spoilage in each half-case lot (Table IV), the only difference in treatment being the time each case was stored under alternating and constant conditions of temperature and humidity (Table I), suggests that interior spoilage is hastened when both temperature and humidity are high. This observation is substantiated by other investigations (2, 4).

The wearing of clean dry gloves whenever eggs are to be handled is a practice which can be recommended. There is no doubt that a reduction in egg spoilage losses could be effected by a more rigid application of recognized sanitary practices both by the producer and in the trade.

Acknowledgments

Grateful acknowledgment is made to the Poultry Division, Central Experimental Farm, Ottawa, especially to Mr. G. Robertson and Dr. S. S. Munro who made arrangements for supplying day-old eggs. The author wishes to thank Dr. J. W. Hopkins, Statistician, National Research Laboratories, for statistical advice.

References

1. FISHER, R. A. and YATES, F. Statistical tables for biological, agricultural and medical research. Oliver and Boyd, Ltd., Edinburgh and London. 1938.
2. GIBBONS, N. E., FULTON, C. O., and HOPKINS, J. W. Can. J. Research. In press.
3. HAINES, R. B. and MORAN, T. J. Hyg. 40(4) : 453-461. 1940.
4. REEDMAN, E. J. and HOPKINS, J. W. Can. J. Research. In press.
5. ROSSER, F. T., WHITE, W. H., WOODCOCK, A. H., and FLETCHER, D. A. Can. J. Research, D, 20(3) : 57-70. 1942.

PRESERVATION OF EGGS

III. STUDIES ON THE EFFECT OF OIL TREATMENT AND EGG-CASE LINER BAGS IN THE PRESERVATION OF SHELL EGGS UNDER ADVERSE CONDITIONS[1]

By E. J. Reedman[2] and J. W. Hopkins[3]

Abstract

Three replicate lots of eggs, each divided into half-case (15 doz.) sets on the treatments used, were stored at 70° F. and 90% relative humidity for 35 days. Different sets of eggs were conditioned at 32°, 40°, 60°, and 70° F. before being placed in the humidity chamber, but no resulting effect of initial condensate on the eggs and packaging material was demonstrated.

Under the conditions of this experiment, oil treatment by the best commercially applicable method, followed by packaging in sealed egg-case liner bags, was found to retard greatly the development of internal defects and severe external mould. Oil treatment alone was definitely beneficial, but the use of egg-case liner bags on untreated eggs was detrimental. While these experiments show that the method of oil treatment combined with bagging was efficacious in these laboratory scale tests, the results obtained from three small test shipments to Great Britain were not in agreement, and indicated that somewhat different storage conditions may be encountered in the export of shell eggs in non-refrigerated holds. The length of time of storage may also be expected to influence directly the degree of spoilage.

Differential spoilage of eggs located in the central and top and bottom trays within the case, and at the periphery and in the interior of individual trays, was also noted under certain conditions. This is considered to indicate the importance of humidity gradients within the case.

Introduction

In the storage and marketing of perishable foods such as shell eggs, a major consideration is protection against spoilage by micro-organisms. The natural defences of shell eggs are not sufficient to prevent spoilage when they are subjected to conditions of temperature and humidity favourable for the growth of moulds and bacteria. The object of this investigation was to make a laboratory study of the efficacy of three commercially practicable treatments which might be used to inhibit spoilage and conserve quality attributes in shell eggs handled under adverse conditions.

The treatments used were oil dipping, using a type of oil and method of application known to be most satisfactory for the test and conditions, oil treatment used in conjunction with packaging in sealed egg-case liner bags, egg-case liner bags used alone with untreated eggs, and untreated eggs as controls. A factor considered to contribute to spoilage in eggs trans-shipped commercially from a cooler to a warmer atmosphere, is the formation of condensate when the humidity is high. Each treatment was accordingly tested under four simulated "handling conditions", giving a graded series of

[1] *Manuscript received September 2, 1942.*
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Published as paper No. 81 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 1086.
[2] *Biochemist.*
[3] *Statistician.*

degrees of condensate on the surface of the eggs and packaging material when trans-shipped to the storage room. The temperature and relative humidity of the storage room were maintained at 70° F. and 90%, respectively, throughout the experiment.

While treatment of shell eggs by immersion in oil, and subsequent drainage of excess oil prior to packaging, has been used to a considerable extent in commercial storage (3, 4), little information is available on the effect of oil treatment at higher storage temperatures. The oil employed in these experiments was a low pour-point paraffin base mineral oil having a Saybolt viscosimeter number of 50/60 at 100° F. This type of oil has been used in commercial storage, and experiments had shown it to be generally superior to heavier oils and waxes for the purpose of this study. The egg-case liner bags used were constructed of two layers of Kraft paper laminated with asphalt, and all were sealed to restrict contact of outside air with the contents.

Experimental

Source of Eggs

Three replicates of the experimental treatments were made, using Grade A Large eggs secured from three central grading stations during the month of April. Grading included candling to obtain as uniform experimental material as possible. White-shelled eggs were used throughout to eliminate the necessity of distribution as to shell colouring in the test lots. Shell porosity could not be measured objectively, but it was possible to choose eggs as nearly alike as could be judged by shell formation. None of the eggs obtained were more than 24 hr. old.

Mould and bacterial load of the eggs as received could not be determined by any commercially practicable method, but variation was minimized by obtaining eggs as nearly new-laid as possible. In view of the fact that all eggs were secured from the grading stations within 24 hr. of their delivery from nearby farms, it is possible that the degree of mould infection at the time of treatment was lighter (2), and the test of treatment correspondingly less severe, than would normally be the case in commercial practice. It is generally realized that oiling or other similar methods of treatment must be applied to fresh eggs subjected to no major contamination, unless some reduction in the microbial flora of older eggs is feasible.

Treatment

After grading, the eggs selected for experimentation were immediately transported to the laboratory by truck, and were conditioned to laboratory temperature before treatment. By use of commercially available egg lifting trays the eggs were removed from the cases, oil dipped, drained, and replaced without touching by hand. Untreated eggs were repacked after conditioning to the same degree as treated eggs.

As earlier findings had indicated decreased penetration of oil when using cool eggs dipped in a heated oil, it was found most convenient to condition all eggs to 80° F. and to dip them in oil conditioned to the same temperature. Under these conditions a 10 sec. immersion and five-minute drainage time (which could be reduced to one minute in commercial practice) were found by experimentation to be adequate, and were adopted throughout. The "small ends" of the eggs were wiped over a brush to remove any excess oil which would not drain off the eggs on standing in the egg lifting tray.

Preliminary tests were made to determine whether treating successive lots of eggs with the same egg dipping oil would result in the oil becoming infected with organisms. After dipping 120 doz. eggs in a 3 gal. volume of oil there was found to be no appreciable increase in microbial content as compared to unused oil. Examination for moulds and bacteria was made by 70° F. incubation of 1 ml. samples with beef peptone and potato dextrose agars for five and four days respectively.

The egg cases used were of the standard ventilated export type having a capacity of 30 doz. The experimental unit was 15 doz. placed in one compartment of each case. To eliminate any variation caused by contamination by fillers and cases, these were sterilized before use. Commercial Keyes trays were used throughout in packaging. In all, three replicates of four experimental sets through four handling procedures required the use of 48 half-case lots of eggs. Each treatment was thus tested on 12 half-case lots under the total of four handling procedures.

Handling and Storage

After treatment, the eggs (including control eggs and untreated eggs in egg-case liner bags, which were simply repacked) were placed in the conditioning chambers prior to storage in the humidity room at 70° F. One half-case set of each treatment in each replicate lot was conditioned to 32° F., one to 40° F., one to 60° F., and one to 70° F. After conditioning, the eggs were moved immediately, through a cool dry atmosphere, to the insulated storage test room at 70° F. and 90% relative humidity. In this way graded amounts of condensate, from a maximum to a minimum were obtained initially on the shells of the eggs and on the fillers and cases.

The storage room was stacked full of cases at all times, empty cases being used as necessary to ensure a constant condition in this regard. The room was ventilated by an extremely slow movement of air through the floor, and the cases containing the experimental eggs were stacked in an objectively random order. Material from previous experiments on egg storage had resulted in a high degree of mould contamination in the storage room, but

plates of potato dextrose agar, exposed in various parts and at various levels, showed a uniformity of mould distribution in the air in which the eggs were stored.

Several pilot half-case lots receiving the various treatments were stored at the same time and examined at intervals. This permitted the gauging of the progress of deterioration. The experimental eggs in the three replicate series were examined only once, after a storage period of 35 days. Thus contamination caused by disturbing the eggs was eliminated, while observations on the pilot lots were indicative of a condition suitable for comparison of treatment.

Examination

The experimental eggs were first examined individually for evidence of external mould growth, and then candled for internal mould and spot rot. The incidence of each type of spoilage was noted separately, and graded visually as either "mild" or "severe". The location and condition of every egg in each tray was recorded on a mimeographed diagram.

External examination was made in a strong light which enabled the detection of very mild growth such as the "whisker" growth of *Mucor*.

Internal moulds show up readily on candling, and may appear as minute black, green, or red spots, or may cover an appreciable area inside the shell. In the present investigation, many instances of mould inside the air cell were found. In some eggs it was difficult to determine if an observed small spot was actually due to mould growth, or was merely a speck of foreign material within the layers of the shell. Such eggs were broken open and a careful examination made.

Moulds do not normally penetrate into the yolk of the egg except in advanced stages of deterioration. Yolk spoilage is generally caused by bacterial growth, sometimes known as spot rot. Spot rot may vary in degree from small spots to a condition turning the whole interior of the egg into a putrid mass. For the purpose of this study the degree of spot rot was taken as "mild" when a spot of the most minute character could be distinguished. Brown and black *Proteus* rots were observed to be most common, but red *Pseudomonas* also occurred.

Results

The design of the experiment was such that all observed percentages could be treated statistically by the analysis of variance procedure, and the significance of any apparent difference was therefore assessed in this way. A

primary analysis was made of the effect of handling and treatment on half-case aggregates, and a secondary analysis of the extent to which treatment effects were modified by the location of the eggs within the half-case.

Lot Averages

Table I shows the average percentage of defects observed at the conclusion of storage in eggs from each of three sources. Significant differences, particularly in respect of internal contamination, are evident. As was noted above, however, treatment comparisons are balanced in respect of such average differences, which may therefore be regarded as an advantage in that they provide a broader basis for the treatment averages.

TABLE I

PERCENTAGE INCIDENCE OF DEFECTS IN EGGS FROM THREE SOURCES AFTER STORAGE FOR 35 DAYS AT 70° F. AND 90% R.H.* (AVERAGE OF ALL TREATMENTS AND HANDLING CONDITIONS)

Defect	Source I	Source II	Source III
External mould, mild or severe	97.9	99.8	100.0
External mould, severe	38.1	42.3	45.8
Internal mould, mild or severe	27.1	35.4	47.6
Internal mould, severe	1.2	10.1	22.4
Spot rot, mild or severe	3.6	6.1	8.8
Any internal defect (mould, spot rot, or both)	30.5	40.9	55.0

* *R.H. = relative humidity.*

Effect of Handling Conditions on Half-case Aggregates

Under the conditions of this experiment the imposed differences in handling procedure had no statistically significant effect on the percentage of external mould, internal mould, or spot rot at the conclusion of storage in either treated or untreated half-case aggregates. The average percentages of severe external mould observed in all the half-cases conditioned at 32°, 40°, 60°, and 70° F. were 47, 42, 35, and 44% respectively, whilst the corresponding figures for internal defects (mild or severe) were 47, 42, 41, and 39%. It is possible that the high temperature and humidity of the storage room resulted in too severe a condition to allow marked differences between the effects of various amounts of condensate.

Effect of Oiling and Bagging on Half-case Aggregates

The percentages of defects recorded after 35 days' storage following each treatment are set forth in Table II. These percentages are each computed from 12 half-case lots (three replicates of each of four handling conditions) containing a total of 180 doz. eggs.

TABLE II

PERCENTAGE INCIDENCE OF DEFECTS IN EGGS AFTER STORAGE FOR 35 DAYS AT 70° F. AND 90% R.H. FOLLOWING VARIOUS TREATMENTS (AVERAGE OF THREE REPLICATIONS OF EACH OF FOUR HANDLING CONDITIONS)

——	Control (untreated)	Oil only	Bags only	Oil and bags
External mould, mild or severe	98.3	99.9	100.0	98.6
External mould, severe	40.9	30.3	96.1	1.1
Internal mould, mild or severe	51.2	30.8	63.5	1.2
Internal mould, severe	15.8	2.2	26.8	0.1
Spot rot, mild or severe	10.7	1.1	12.8	0.1
Any internal defect (mould, spot rot, or both)	58.5	31.6	77.2	1.3

Practically all eggs showed some external mould, either mild or severe, irrespective of treatment. The incidence of severe external mould was, however, significantly affected by oiling and bagging. Oiling without bagging caused a reduction of about 10% in comparison with the untreated controls. Oil treatment in the absence of a mycostatic agent cannot be expected to reduce the incidence of external mould appreciably, and its effect is conditioned by the type of eggs and the conditions of storage (2). Bagging without oiling clearly favoured growth, and led to an increase of 55% in severe external mould. On the other hand the combination of oiling with bagging proved to be very effective, and restricted severe external mould to about 1% of the eggs treated in this way.

Total internal mould (mild or severe) was reduced by about 20% following oiling alone, but was appreciably further restricted (to about 1%) by the combination of oiling and bagging. Bagging without oiling was markedly detrimental. Similar tendencies manifest themselves in the recorded percentages of severe internal mould, although oiling alone was practically as effective as oiling and bagging. Spot rot was likewise effectively inhibited by oiling, but was not significantly affected by bagging either alone or in conjunction with oiling.

Observations based on size of air cell, yolk index, and hydrogen ion concentration of the white showed that at the conclusion of storage the average quality of the untreated eggs and those bagged without oiling was poor. That of the oiled eggs was considered to be good, and that of those oiled and bagged very good.

Influence of Position in Half-case

As was mentioned above, practically all eggs developed some degree of external mould. There were, however, appreciable differences in the incidence of severe external mould in both untreated and oiled eggs ascribable to location within the case, as illustrated in Table III. Both these types of eggs were subject to less severe external mould in the top and bottom trays than those in the middle of the case. On the other hand the percentage of this defect in eggs bagged without oiling was uniformly high in all trays, and in those both oiled and bagged, uniformly low.

TABLE III

PERCENTAGE OF SEVERE EXTERNAL MOULD AFTER STORAGE FOR 35 DAYS AT 70° F. AND 90% R.H. IN EGGS CLASSIFIED ACCORDING TO TREATMENT AND LOCATION IN CASE (AVERAGE OF THREE REPLICATIONS OF EACH OF FOUR HANDLING CONDITIONS)

Location	Control (untreated)	Oil only	Bags only	Oil and bags
First tray (top)	11.1	30.9	93.4	3.9
Second tray	49.6	37.6	96.4	0.6
Third tray	55.3	39.0	97.5	1.4
Fourth tray	53.5	41.0	98.6	0.0
Fifth tray	48.0	26.7	96.9	0.3 Nec. diff.
Sixth tray (bottom)	16.0	8.0	96.1	0.3 = 9.2
Inside rows (all trays)	56.0	40.2	99.5	1.7 Nec. diff.
Outside row (all trays)	27.5	24.1	94.3	0.6 = 5.4

The controls and the eggs oiled without bagging also exhibited a lower frequency of severe external mould if located in the outside row, rather than in the central portion of a Keyes tray. This differential varied somewhat from tray to tray, but was appreciable in the untreated eggs subjected to all four "handling" temperatures (Table IV), whereas in oiled eggs it was definitely most pronounced following "handling" at 32° F., and was statistically insignificant in the cases handled at 60° F., and 70° F. Severe external mould was

TABLE IV

PERCENTAGE OF SEVERE EXTERNAL MOULD AFTER STORAGE FOR 35 DAYS AT 70° F. AND 90% R.H. IN EGGS "HANDLED" AT 32°, 40°, 60°, AND 70° F. (AVERAGE OF THREE REPLICATIONS)

——	32° F.	40° F.	60° F.	70° F.
Untreated				
Inside rows (all trays)	67.9	44.4	45.7	66.2
Outside rows (all trays)	32.1	25.9	19.5	32.7
Oil only				
Inside rows (all trays)	70.6	38.9	14.4	36.9 Nec. diff.
Outside row (all trays)	34.0	24.2	8.9	29.3 = 10.8

uniformly high in both the inner and outer rows of trays of eggs bagged without oiling, and in those both oiled and bagged it was uniformly infrequent irrespective of position.

Internal defects were likewise less frequent in the bottom trays of the cases containing eggs untreated, oiled only, or bagged without oiling, as indicated in Table V. The untreated eggs also showed fewer of these defects in the top

TABLE V

PERCENTAGE OF INTERNAL DEFECTS (MILD OR SEVERE MOULD, SPOT ROT, OR BOTH) AFTER STORAGE FOR 35 DAYS AT 70° F. AND 90% R.H. IN EGGS CLASSIFIED ACCORDING TO TREATMENT AND LOCATION IN CASE (AVERAGE OF THREE REPLICATIONS OF EACH OF FOUR HANDLING CONDITIONS)

—	Control (untreated)	Oil only	Bags only	Oil and bags
First tray (top)	55.0	35.8	68.1	2.3
Second tray	59.6	30.8	71.3	2.0
Third tray	65.0	30.5	69.2	0.6
Fourth tray	64.4	34.3	68.0	1.1
Fifth tray	61.4	35.4	64.9	0.6 Nec. diff.
Sixth tray (bottom)	44.3	23.4	61.7	0.9 = 6.6
Inside rows (all trays)	67.5	30.5	85.5	1.4 Nec. diff.
Outside rows (all trays)	52.2	32.5	55.0	1.1 = 3.8

than in the middle trays, but this is not true of the treated eggs. On the average there was a significantly higher percentage of internal defects in control eggs and in those bagged without oiling when located in the interior of the tray than when at the outside (Table V). This effect was, however, a function of "handling" temperature, and is accordingly analysed in more detail in Table VI. From this tabulation it is to be seen that the controls

TABLE VI

PERCENTAGE OF INTERNAL DEFECTS (MILD OR SEVERE MOULD, SPOT ROT, OR BOTH) AFTER STORAGE FOR 35 DAYS AT 70° F. AND 90% R.H. IN EGGS "HANDLED" AT 32°, 40°, 60°, and 70° F. (AVERAGE OF THREE REPLICATIONS)

—	32° F.	40° F.	60° F.	70° F.
Untreated				
Inside rows (all trays)	73.1	71.4	61.2	64.3
Outside row (all trays)	56.3	52.5	49.7	50.2
Oil only				
Inside rows (all trays)	50.4	34.2	19.7	17.7
Outside row (all trays)	41.8	31.3	31.1	25.9
Bags only				
Inside rows (all trays)	80.7	85.3	88.4	87.6
Outside row (all trays)	70.5	45.9	53.0	50.7
Oil and bags				
Inside rows (all trays)	4.2	0.0	1.3	0.0 Nec. diff.
Outside row (all trays)	2.6	0.0	1.6	0.3 = 7.6

showed such a differential following all four handling temperatures. So did the eggs bagged without oiling, but here the difference was definitely less pronounced in those cases handled at 32° F. than in those subjected to any of the other three handling temperatures. It is also noteworthy that the eggs oiled without bagging showed more defects at the outside than in the interior of the trays following handling at 32° F., but less after handling at 60° and 70° F.

Discussion

It is evident from Table I that different lots of eggs may develop appreciably different amounts of spoilage during storage. The three lots of eggs were all taken at one season of the year, and indicate variation between eggs gathered from different areas throughout a small section of Ontario. Further variation between seasons has been observed in other experiments (1). Since the amount of spoilage may be determined to a considerable extent by contamination prior to treatment and storage, the success of oil treatment in practice may well depend on its application early to relatively uncontaminated eggs (2).

Oil treatment of shell eggs prevented spoilage to an appreciable extent, but was not as effective as oil treatment followed by packing in egg-case liner bags under the conditions of this test. Bagging without oiling was clearly detrimental, probably because of saturation of the atmosphere within the bags by water vapour diffused from the eggs themselves. Oil treatment, if properly applied, retards the loss of moisture from eggs and this was possibly responsible for the difference obtained in the use of the egg-case liner bags.

Some indication of the effect of physical conditions on the development of micro-organisms may be seen from Tables III to VI. The imposed differences in prestorage handling temperature were designed to illustrate the effect of condensate from the external atmosphere. The results show that the degree of condensate was apparently not the main factor responsible for the differential spoilage of eggs at the centre and periphery of the cases, as this occurred in the absence of condensate. It clearly had some effect in modifying this differential in eggs which were not enclosed in egg-case liner bags. Maintenance of a steady storage temperature is considered to have favoured the bagging treatments by preventing condensation after storage for a length of time in which moisture could have accumulated within the bags.

Acknowledgments

The assistance of members of the staff of the Division of Applied Biology, National Research Council, and of the Poultry Marketing and Inspection Services, Department of Agriculture, facilitated this study.

References

1. REEDMAN, E. J. Unpublished data. 1941.
2. ROSSER, F. T. Can. J. Research, D, 2 (10) : 291-296. 1942.
3. SWENSON, T. L. Food Research, 3 : 599-608. 1938.
4. UNITED STATES DEPARTMENT OF AGRICULTURE, Circular No. 583. 1941.

PRESERVATION OF EGGS

IV. STORAGE OF OILED AND BAGGED EGGS UNDER CONSTANT AND ALTERNATING TEMPERATURES AND HUMIDITIES[1]

By N. E. Gibbons[2], C. O. Fulton[2], and J. W. Hopkins[3]

Abstract

Untreated eggs and eggs oiled and sealed in egg-case liner bags were stored for six weeks in standard export cases (*a*) at 70° F. and 90% relative humidity continuously, (*b*) at 65° F. and 95% relative humidity alternating with 75° and 70% every two days, the dew point consequently always remaining below 65° F., and (*c*) at 60° F. alternating with 80° every three days, both at 90% relative humidity, but the dew point varying from 57° to 77° F. respectively. Spoilage was least under the second set of conditions and greatest under the third. The indications were that within rather wide limits, microbiological development was dependent primarily upon storage conditions rather than upon the quantity of inoculum on the surface of the eggs at the beginning of storage. The bags used had little effect on intracase temperatures.

Under conditions (*a*) and (*c*) oiling and bagging reduced internal mould and rot but did not significantly affect the development of external mould; under conditions (*b*) internal spoilage was uniformly low in both treated and control eggs. All oiled and bagged eggs were in better physical condition after storage than were the untreated, having smaller air cells, freer yolks, and less distinct yolk shadows. Variations in the incidence of spoilage within cases occurred and are attributed to intracase temperature and humidity differentials.

Introduction

In a previous study in these laboratories (1) it was observed that oiling followed by enclosure in egg-case liner bags appreciably reduced mould and bacterial spoilage of eggs stored for six weeks at 70° F. and 90% relative humidity. Subsequent trial overseas shipments were not, however, productive of similar findings, from which it appeared that the foregoing experimental storage conditions might not be a suitable substitute for those typically encountered in shipping. The two additional experiments now reported were accordingly undertaken in order to accumulate further information respecting the influence of environmental conditions on the efficacy of this mode of treatment of shell eggs.

In the first of these, treated and control eggs characterized by three levels of initial bacterial and mould contamination of the shell were subjected to cyclic variations in temperature and humidity in which two days at 65° F. and 95% relative humidity alternated with two days at 75° F. and 70% relative humidity successively for six weeks. The relative humidity was thus higher in the low phase of the temperature cycle, and *vice versa*, but the dew

[1] *Manuscript received September 2, 1942.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 1087 and as Paper No. 82 of the Canadian Committee on Food Preservation.

[2] *Bacteriologist, Food Storage Investigations.*

[3] *Statistician.*

point remained practically constant. In the second test a temperature differential of 20° F. was imposed, three-day periods at 60° alternating with three days at 80° F., again for six weeks. The relative humidity was maintained at the high level of approximately 90% throughout and consequently the dew point varied from 57.2° F. to 77° F. at temperatures of 60° and 80° F. respectively. For comparative purposes, the previously employed constant storage conditions of 70° F. and 90% relative humidity were also repeated in both tests.

Experimental

Eggs

All eggs used were obtained from one eastern Ontario grading station in January and April. White-shelled Grade A Medium eggs were specified, but for the first experiment it was necessary to accept four cases (out of 18) of Grade A Pullet eggs. The contents of all cases were accordingly redistributed so as to include whole trays of pullet eggs in random order in each case. Subsequent analyses of variance showed that these did not differ significantly from the medium eggs in respect of either internal or external spoilage.

Three replicate lots each comprising six 30-doz. cases or 12 15-doz. experimental units were employed in the first experiment. In the second experiment three lots of three cases each were used. These were disposed so as to provide six replicates of each 15-doz. experimental unit under alternating and three under constant storage conditions.

Shell Contamination

In the first experiment three levels of initial shell contamination were introduced. To provide these, two-case lots of each replication were dipped in a disinfectant solution of 3% U.S.P. hydrogen peroxide containing $\frac{1}{5}$ gr. acetanilid per oz., two were left untreated, and the remaining two were dipped in a saline suspension of bacteria and fungi. This last contained a mixture of representative types of organisms isolated from bad eggs, growths from two-day old plate cultures of bacteria and week-old cultures of fungi being washed off and diluted with saline solution to give a just cloudy suspension. Bacterial counts at the time of dipping the first, second, and third replications were 3,500,000, 2,100,000, and 66,500,000 per ml. respectively. Before the dipping of the second replication the mould count was 150,000 per ml., but no moulds appeared in a 1 : 10,000 dilution made prior to dipping the third replication. In the interval between dipping replicates, the suspension was maintained at 45° F., but on each occasion was brought into the laboratory the night before being used and was allowed to come to room temperature.

By means of commercially available egg lifters, tray lots were dipped simultaneously, the eggs being immersed for one minute and drained for 30 sec. After draining, peroxide dipped eggs were transferred at once to sterilized Keyes trays and left at laboratory temperature. Those dipped in the suspension of organisms were returned to their original trays and while

still wet placed in a room maintained at 30° F., on the assumption that sudden chilling might draw bacteria and mould spores through the shell. Four to five hours later they were returned to the laboratory and left along with the peroxide dipped and untreated eggs until the next day, when half of each case lot was oiled and bagged.

Throughout this period, laboratory temperature was about 25° C. (77° F.).

Oiling and Bagging

All eggs in both experiments were left at laboratory temperature overnight prior to the oiling and bagging of half of each case (selected at random). Control (unoiled and unbagged) eggs were transferred to sterile Keyes trays where necessary and placed in the appropriate half of a sterilized export case. In the first experiment oiling and packing were done in the order: peroxided, untreated, and contaminated. In both experiments, eggs were dipped for 10 sec. in a mineral oil of the same composition as used previously (1) (fresh oil being used for each replication) and drained for at least three minutes. The drop of oil remaining on the small end of each egg after drainage was removed with an oiled brush, following which bagging took place immediately. The bags used were of the asphalt laminated egg-case liner type employed previously, the tops of which were folded over and securely fastened with gummed paper and cellulose tape.

Storage

One-half of the eggs (nine cases) in the first experiment and one-third (three cases) of those in the second were again kept under the constant storage conditions previously employed, whilst the remainder were subjected to alternating temperatures and humidities designed to simulate and even to exaggerate changes during transport. Once all cases had been placed in the storage rooms the position of each was changed every four days during the first experiment according to a random scheme, the cases being turned end for end with each change. During the second experiment, the position of the cases was changed every week.

During the first experiment, the average temperature over the whole storage period in the constant condition room was 70.5° F. and the average relative humidity, 91.5%. The highest temperature recorded was 72.5°, but on two occasions readings of 60.5° and 61.5° F. respectively, occurred as a result of pump and fan failures. Relative humidity fluctuations were confined to the range 83 to 95%. In the alternating condition room the change from one set of conditions to the other was usually completed within

three to four hours (Fig. 1). There were greater uncontrolled fluctuations in this than in the former room, but the recorded extremes of temperature and humidity were 59° and 86° F., and 58 and 100% relative humidity, respectively. The averages, over the whole experiment, of the first phase of the

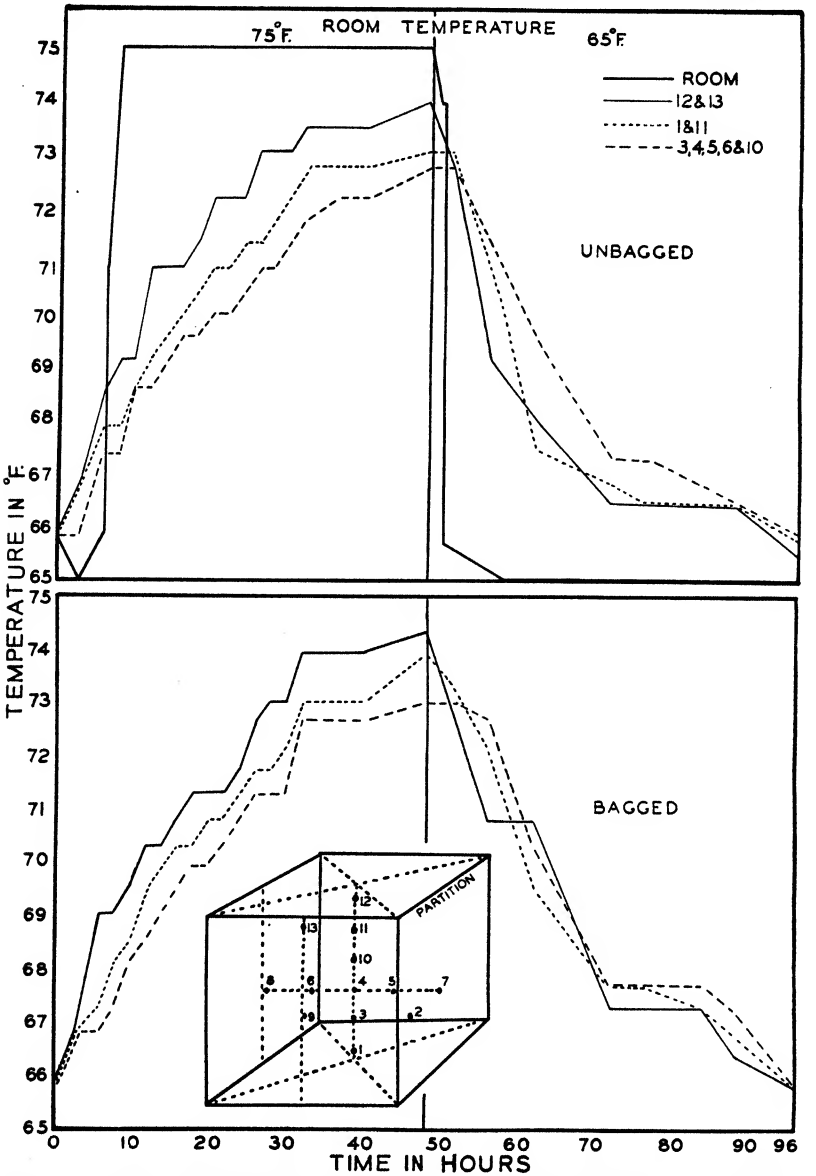


FIG. 1. *Change of temperature in egg cases following changes in external temperature from 65° to 75° F.*

temperature and humidity cycle were 63.7° F. and 95.7% relative humidity, and of the second 74.4° F. and 69.6% relative humidity.

In the second experiment, the averages for the constant condition room were 70.7° F. and 92.4% relative humidity. The average temperature in the high phase of the cycle in the alternating room was 80.0° F., but there were greater irregular fluctuations than previously, and a minimum of 50° and a maximum of 110° F. were recorded for short periods. The average temperature for the low phase was 62.4° F., although again extremes of 45° and 97° were recorded for brief intervals. Relative humidity was on the average 87.3% at the high and 86.1% at the low temperature.

Measurements of Intracrate Temperature

To secure measurements of intracrate temperatures, copper-constantan thermocouples were installed in one of the crates stored in the alternating room during the first experiment. These were fastened with tape to points on the various trays as indicated in Fig. 1, corresponding points being selected on each side of the central position, i.e., in the bagged and unbagged half of the crate. In the former, the thermocouple leads were brought out through the top of the bag and the bag sealed with hot asphalt.

Examination of Eggs

After six weeks of storage all eggs were individually examined under a strong light for signs of external mould, and were then candled (and in any doubtful instance broken out) for the recording of internal defects. No attempt was made to distinguish between internal mould and rots. Stuck yolks, jelly spots, and tiny mould colonies were recorded as mild, and all definite mould colonies and rots as severe.

Results

Intracrate Temperature Alternations

Observations were made at intervals over two complete temperature cycles when the experimental crate was in different positions in the room, but as the results for both were similar, the changes during one cycle only are shown in Fig. 1. As would be anticipated there was an appreciable lag in both the uptake and loss of heat by the crate, and actually the internal temperature in both the bagged and unbagged portion did not quite reach equilibrium with that of the surrounding air before the next phase of the cycle began. The bag appeared to exert no appreciable effect on the rate at which the internal temperature rose, but retarded cooling somewhat. In both the bagged and unbagged half of the crate the first (top) tray responded most rapidly to external changes, and the second and bottom ones were next, while the middle trays showed the greatest lag. The central group of thermocouples (3, 4, 5, 6, and 10 in Fig. 1) usually exhibited a range of less than 1° F. No. 13, located at the edge of the top tray, was usually about half a degree ahead of No. 12 (top centre), but this differential was not observed in the middle and lower

parts of the crate. The two top trays most nearly approached room temperature, coming to within about a degree of the upper and about half a degree of the lower level. In the case of the other trays the discrepancy was of the order of one degree at the termination of both the high and low phases.

TABLE I

AVERAGE PERCENTAGE OF EGGS SHOWING EXTERNAL MOULD AND INTERNAL DEFECTS (FIRST EXPERIMENT)

Treatment	Severe		Mild + severe	
	65° F., 95% R.H.* and 75° F., 70% R.H. alternating	70° F., 90% R.H. continuously	65° F., 95% R.H. and 75° F., 70% R.H. alternating	70° F., 90% R.H. continuously
	External mould			
Oiled and bagged	0	19.1	2.5	59.1
Control	0	11.3	1.9	68.7
	Internal defects			
Oiled and bagged	2.6	2.7	4.5	21.8
Control	4.5	51.6	6.2	66.6

**R.H.=relative humidity.*

External Mould in Half-case Aggregates

The average percentages of external mould recorded under the various conditions of the first experiment are shown in Table I, whilst Table II summarizes the results of an analysis of variance of the data for individual half-cases.

TABLE II

ANALYSIS OF VARIANCE OF PERCENTAGE OF EXTERNAL MOULD AND INTERNAL DEFECTS IN EGGS STORED UNDER CONSTANT AND ALTERNATING CONDITIONS (FIRST EXPERIMENT)

Source of variance	D.f.	Mean square, exterior mould		Mean square, interior defects	
		Severe	Mild + severe	Severe	Mild + severe
Lots	2	1176.84*	3450.48**	731.89	2126.51*
Shell contamination	2	197.69	698.79	601.51	1042.43
Storage conditions	1	2076.32**	34249.67**	4998.49**	13564.48**
Treatment	1	136.11	180.45	5816.61**	4867.39**
Shell × storage	2	197.70	202.60	321.50	545.82
Shell × treatment	2	34.23	350.06	291.55	129.55
Storage × treatment	1	136.11	233.07	4974.95**	4177.47**
Shell × storage × treatment	2	34.22	342.39	362.44	270.64
Error	22	260.29	470.27	284.46	343.49

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

The constant conditions of 70° F. and 90% relative humidity were much more favourable for the growth of mould on the outside of the shells than were the alternations of 65° and 95% and 75° and 70%. Under the latter in fact no severe and only a slight amount of mild external mould developed. There was a statistically significant difference between the three replicate lots of eggs, the third being most susceptible to spoilage of all kinds, but the design of the experiment was such that this affected all treatment comparisons equally. Oiling and bagging was without apparent effect on the development of external mould under either set of conditions, nor was any influence of the level of initial shell contamination demonstrable.

TABLE III

AVERAGE PERCENTAGE OF EGGS SHOWING EXTERNAL MOULD AND INTERNAL DEFECTS (SECOND EXPERIMENT)

	Severe		Mild + severe	
	60 – 80° F., 90% R.H.	70°F., 90% R.H. continuously	60 – 80° F., 90% R.H.	70° F., 90% R.H. continuously
	External mould			
Oiled and bagged	15.0	2.4	76.9	94.6
Control	30.9	1.7	93.0	77.4
	Internal defects			
Oiled and bagged	9.1	1.5	24.1	8.3
Control	80.5	44.4	96.0	72.8

Since in the second experiment only three cases were stored under constant and six under alternating conditions, separate analyses of variance of the two series of results are given in Table IV. Following the alternating conditions

TABLE IV

ANALYSIS OF VARIANCE OF PERCENTAGE OF SPOILAGE IN EGGS STORED UNDER ALTERNATING AND CONSTANT CONDITIONS (SECOND EXPERIMENT)

Source of variance	D.f.	Mean square, external mould		Mean square, internal defects	
		Severe	Mild + severe	Severe	Mild + severe
Alternating conditions					
Replicates	5	1102.6*	293.3*	164.7	252.6
Treatments	1	761.6	776.0**	15279.6**	15508.8**
Error	5	136.3	45.4	77.7	180.5
Constant conditions					
Replicates	2	0.882	151.2	151:3	11.4
Treatments	1	0.807	443.8	2769.2*	6233.9*
Error	2	9.882	48.7	134.8	121.8

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

of this test there was significantly less external mould on the oiled and bagged than on the control eggs, but under the constant conditions the observed differences between treatments were not statistically significant considering the small number of cases employed (Table III).

Internal Defects in Half-case Aggregates

When the eggs were being candled after storage it was noted that the oiled and bagged ones were for the most part in better internal condition than the controls, having smaller air cells, freer yolks, and less distinct yolk shadows. Of the "gradeable" eggs, most of those oiled and bagged would grade B, whilst the unbagged ones were of Grade C.

In the first experiment (Tables I and II) the incidence of internal defects under the alternating conditions imposed was uniformly low irrespective of the level of initial shell contamination and of whether the eggs were oiled and bagged or not. Under the constant conditions however, much more internal spoilage developed in the control than in the oiled and bagged eggs. As in the case of external mould, there were significant differences in the average amount of spoilage in the replicate lots of eggs, but again no significant effect of the artificial modifications of initial shell contamination although the observed amount of spoilage increased in the order: peroxided, control, and contaminated eggs.

A beneficial effect of oiling and bagging in reducing internal spoilage was also observed in the second experiment (Tables III and IV), but in this case following alternating as well as constant storage conditions.

Incidence of Spoilage in Relation to Position of Egg in Case

From the previous study (1) there was some indication of differential spoilage of the eggs located on the outside and inside rows of the Keyes trays. In considering this point in the present investigation, eggs adjacent to an end or side of the crate were classified as "outside", whilst those adjacent to the central partition were classed together with those in the central portion of the trays as "inside".

Table V gives the average percentages of inside and outside eggs showing spoilage in the first experiment. Under the alternating storage conditions of this test the incidence of both external and internal growths was uniformly low in both oiled and bagged and control eggs, irrespective of their position in the tray. Under constant temperature and high humidity conditions on the other hand a more frequent occurrence of both external and internal spoilage in the central portion of the trays was statistically demonstrable for control eggs, whilst the effect of oiling and bagging was not only to reduce the total amount of deterioration as already noted, but also to eliminate the foregoing differential between the inside and outside rows of the trays.

TABLE V

AVERAGE PERCENTAGE OF SPOILAGE IN INSIDE AND OUTSIDE ROWS OF TRAYS (FIRST EXPERIMENT)

—	Severe		Mild + severe	
	65° F., 95% R.H. and 75° F., 70% R.H. alternating	70° F., 90% R.H. continuously	65° F., 95% R.H. and 75° F., 70% R.H. alternating	70° F., 90% R.H. continuously
	External mould			
Oiled and bagged				
Inside	0	18.0	3.5	59.4
Outside	0	20.2	1.5	58.9
Control				
Inside	0	14.8	3.5	73.1
Outside	0	7.5	0.3	64.0
Necessary difference between inside and outside	3.8		4.8	
	Internal defects			
Oiled and bagged				
Inside	2.5	2.4	4.2	20.7
Outside	2.7	2.9	4.8	23.0
Control				
Inside	4.3	54.0	6.2	67.3
Outside	4.8	49.1	6.3	65.9
Necessary difference between inside and outside	2.4		3.0	

In the second experiment (Table V) there was likewise more spoilage, both external and internal, of central than of peripheral eggs when they were stored unbagged under the constant conditions of 70° F. and 90% relative humidity, and here again the effect of oiling and bagging was to eliminate this differential. It is noteworthy however that in the other part of this trial, in which humidity was maintained at a high level throughout both phases of the 20° temperature alternations, significantly less spoilage occurred in the outer rows of the oiled and bagged but not of the control eggs, i.e., just the reverse of the condition observed at constant temperature.

No significant effect of the position of tray in the crate on the development of spoilage was noted in the first experiment. In the second experiment, under the alternating temperature and high humidity conditions, there was more external mould in the middle trays of both oiled and bagged and control

eggs than in those located at either the top or bottom of the crate; there was also a tendency for the amount of internal spoilage to be greatest in the top trays and to diminish progressively towards the bottom of the cases. On the other hand, after storage at constant temperature and high humidity, position of the tray affected significantly only total internal defects, more spoilage developing at the bottom of the cases than at the top (the reverse of the trend observed under conditions of alternating temperature and high humidity). These results are shown in Table VI.

TABLE VI

AVERAGE PERCENTAGE OF SPOILAGE IN INSIDE AND OUTSIDE ROWS OF TRAYS (SECOND EXPERIMENT)

	Severe		Mild + severe	
	60 – 80° F., 90% R.H.	70° F., 90% R.H. continuously	60 – 80° F., 90% R.H.	70° F., 90% R.H. continuously
	External mould			
Oiled and bagged				
Inside	22.1	2.9	87.8	95.3
Outside	7.4	1.9	65.1	93.9
Control				
Inside	33.0	2.9	95.0	82.8
Outside	28.7	0.4	90.8	71.6
Necessary difference between inside and outside	13.3	5.0	6.8	4.1
	Internal defects			
Oiled and bagged				
Inside	12.9	1.1	32.3	8.3
Outside	4.9	1.9	15.3	8.4
Control				
Inside	84.9	48.0	96.6	73.8
Outside	75.7	40.6	95.4	71.6
Necessary difference between inside and outside	9.5	6.3	6.3	14.4

pH of White and White Thickness

In the first experiment it was possible to measure the pH and thickness (height) of the white of four eggs (two centrally and two peripherally located) from practically every tray of the oiled and bagged half-cases and of the controls stored under alternating conditions. Owing to almost invariable rupturing of the yolk-membrane, it was not possible to secure measurements

for the controls stored under constant conditions. Observations were thus secured on 215 oiled and bagged and 208 control eggs stored under alternating conditions, and on 213 oiled and bagged eggs stored under constant conditions.

Following storage under alternating conditions the average pH of the oiled and bagged eggs measured (8.63) was significantly lower than that of the controls (9.23). There was no difference between the replicate lots of eggs in this respect, and the manipulations incidental to the production of different degrees of shell contamination were also apparently without effect on the pH at the conclusion of storage.

White thickness was found to be much more sensitive to treatment and environment. After storage under alternating conditions the oiled and bagged eggs had an average white thickness one-third greater than that of the controls. Furthermore, this characteristic was affected by the initial shell treatment. The white of oiled and bagged eggs previously dipped in hydrogen peroxide was significantly thinner than that of those either contaminated or initially untreated; whilst of the controls (not oiled and bagged) the white of the peroxided eggs was significantly thinner than that of those with untreated shells, but did not differ significantly from those dipped in the contaminating suspension (Table VII). A comparison of the results for

TABLE VII

AVERAGE WHITE THICKNESS (IN.) OF EGGS HELD UNDER ALTERNATING CONDITIONS (FIRST EXPERIMENT)

Treatment	Shell condition			Average
	Normal	Reduced	Contaminated	
Oiled and bagged	0.103	0.087	0.106	0.099[1]
Untreated	0.075	0.059	0.063	0.066[1]
Average	0.089[2]	0.073[2]	0.083[2]	

[1] *Necessary difference = 0.008.*
[2] *Necessary difference = 0.009.*

TABLE VIII

AVERAGE WHITE THICKNESS (IN.) OF OILED AND BAGGED EGGS HELD UNDER ALTERNATING AND CONSTANT CONDITIONS (FIRST EXPERIMENT)

	Shell condition			Average
	Normal	Reduced	Contaminated	
Alternating	0.103	0.087	0.106	0.099[1]
Constant	0.093	0.082	0.094	0.090[1]
Average	0.098[2]	0.084[2]	0.100[2]	

[1] *Necessary difference = 0.006.*
[2] *Necessary difference = 0.007.*

oiled and bagged eggs kept under alternating and constant conditions (Table VIII) indicated that the latter, if initially contaminated or untreated, had the thinner whites, whilst following both sets of storage conditions the peroxide dip resulted in a uniformly low value of this characteristic.

Discussion

The failure of the modifications of initial shell contamination imposed in the first of the present experiments to influence subsequent spoilage is noteworthy. From the data available it is impossible to determine whether this was due to the inefficacy of the methods of infection and disinfection adopted, or to the fact that spoilage is, within rather wide limits, dependent primarily upon environmental conditions rather than upon the quantity of inoculum originally present; the latter supposition however receives some support from the findings of Rosser (2) who, using a contaminating liquid containing from 50 to 100 times the number of organisms per unit volume in that employed by the present writers, was able to demonstrate a significant relation between initial contamination and subsequent storage. On the other hand, the adverse effect of peroxide dipping is in accordance with the observations of Rosser *et al.* (3) who noted that this treatment did not preserve either white thickness or yolk index. As oiling and bagging reduced the detrimental action appreciably (Table VII), it seems possible that this effect of the peroxide may have been partially offset by a greater retention of carbon dioxide by the oiled and bagged eggs, reflected in their lower pH.

The results of the two present experiments are in agreement with each other and with the earlier results of Reedman and Hopkins (1) in indicating a significant reduction of internal spoilage by oiling and bagging of the eggs stored under constant conditions of 70° F. and 90% relative humidity. Furthermore, although the degree of control (Tables I and III) fell short of that achieved in the preceding trial, when the incidence of all forms of internal defects was reduced to about 1%, it is also true that the percentage of total internal spoilage in untreated eggs in the two present trials, at 67 and 73%, was about 20% higher than the corresponding 51% occurring previously (1). Part of this increase is no doubt attributable to the extra week the present eggs were stored. External mould, on the other hand, was more pronounced in the preceding experiment than in the two present ones, but was also more amenable to control, even if allowance be made for some divergence of classification as "mild" and "severe" attributable to personal factors. It must be concluded therefore that the results of treatment may be dependent to some extent on the initial history of the eggs dealt with. Rosser (2) obtained his "market" eggs in February and their age was not known. Reedman and Hopkins (1) obtained their supplies in April from grading stations and the eggs were probably only 24 hr. old. In the present tests, in January and April, the ordinary run of grading station eggs, varying from one to seven days old, were used. This difference in age may account for the above mentioned

difference in the amount of spoilage. Since all tests were made when eggs are considered to be of the best quality, the season cannot be considered as a major factor.

In spite of the above incongruities however the discrepancies in spoilage, both external and internal, following storage at 65° to 75° F. and 95 to 70% relative humidity on the one hand, and at 60° to 80° F. and 90% on the other, are of an altogether greater order than those occurring in the two parallel series kept at 70° F. and 90% continuously, and accordingly, may reasonably be considered to result from the conditions of storage rather than from differential susceptibility of the eggs used on the two occasions. It is to be remarked that in all four series the mean storage temperature was approximately 70° F. In the first of the present experiments the alternating conditions were such that the dew point should never be reached and hence condensation should not occur. Owing to some uncontrolled variation, condensation was probably not altogether absent. In the second experiment the situation is complicated by the lag in the thermal and doubtless also in the humidity cycle within the crates relative to that in the surrounding atmosphere. It seems probable, however, that even after allowing for this the temperature of the eggs must have fallen below the dew point at some stage in the progression from 60° to 80° F. Condensation would therefore be expected to have more influence in this than the preceding set of alternating conditions.

The differential spoilage following storage at 70° F. and 90% relative humidity of eggs peripherally and centrally located in the trays, is in accordance with previous experience (1). At the conclusion of storage, condensate was observed in six of the nine cases held under these conditions in the first of the present experiments. It was noted that such condensate was most pronounced in the middle trays and on the eggs located in the centre of these but in view of the fact that this condition occurred in four lots of bagged as compared to three of unbagged eggs, it cannot be related directly to the incidence of spoilage. Whilst variations in spoilage within crates were again encountered in the second experiment, these did not parallel the trends previously observed, and the results as a whole justify only the general conclusion that unequal spoilage may occur, presumably as a consequence of intracrate temperature and humidity differentials such as those noted in the first experiment.

Acknowledgments

The authors wish to thank Dr. W. H. Cook for many helpful suggestions. They gratefully acknowledge the assistance of Mr. R. L. Moore in setting up these experiments and maintaining temperature conditions, of Mr. T. A. Steeves and Mr. E. Chadderton in arranging for the alternating conditions, and of Mr. M. Mitchell of the Poultry Services, Department of Agriculture, for procuring the eggs.

References

1. REEDMAN, E. J. and HOPKINS, J. W. Can. J. Research, D, 20(10) : 297-305. 1942.
2. ROSSER, F. T. Can. J. Research, D, 20(10) : 291-296. 1942.
3. ROSSER, F. T., WHITE, W. H., WOODCOCK, A. H., and FLETCHER, D. A. Can. J. Research, D, 20(3) : 57-70. 1942.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. NOVEMBER, 1942 NUMBER 11

SEARCH FOR SOURCES AND CARRIERS OF EQUINE ENCEPHALOMYELITIS VIRUS[1]

BY RONALD GWATKIN[2] AND IRVIN W. MOYNIHAN[3]

Abstract

Brains of 112 ground squirrels (*Citellus richardsoni*), 10 hawks, one burrowing owl, three pheasants, four crows, nine rats (eight *Mus decumanus* and one *M. rattus*) two wild ducks, two jack rabbits, three hens, three pigs, and two cats were examined for the presence of virus. Spleens of 15 ground squirrels and livers of three others, also spleens of five hawks and six rats were examined. Six assassin bugs (one *Zelus audax* and five *Sinea diadema*), 133 mosquitoes, 30 *Stomoxys* sp., 10 *Tabanidae*, 30 crickets, 64 grasshoppers, and 12 ticks (*Dermacentor andersoni* Stiles) were also examined in suitable sized lots.

Two guinea pigs were injected with a suspension of each lot. If suspicious temperature reactions occurred, these animals were destroyed and suspensions of their brains were injected into other guinea pigs. All the injected animals that survived, regardless of whether or not they had shown any temperature reaction, were given a challenge inoculation of 25 M.L.D. of Western virus three weeks or longer after the first injection.

Lot 21, consisting of two ground squirrel brains, was the only group to give any evidence suggestive of the presence of virus. Virus could not be isolated from this lot but temperatures of the injected guinea pigs, and subsequent resistance of the survivor to a challenge inoculation, were very suggestive of its presence. This is the second occasion on which results suggested the presence of virus in very small amount in ground squirrel brains.

In 1940, Gwatkin and Moore (3) published a report indicating the possible presence of equine encephalomyelitis virus (Western) in a group of ground squirrel brains examined for the virus by guinea pig inoculation. A year later Gwatkin (2) reported negative results from the examination of 200 brains of ground squirrels, mice, and other small mammals and birds, 34 ground squirrel spleens, and 527 ticks (*Dermacentor andersoni* Stiles).

Cox *et al.* (1) recently isolated Western virus from a naturally infected prairie chicken during an epidemic of human encephalitis. Both the blood and central nervous system harboured the virus. Hammon *et al.* (5) reported the recovery of equine encephalomyelitis virus (Western) and St. Louis virus from mosquitoes (*Culex tarsalis*). They (4) carried out neutralization tests on the blood of wild and domestic animals and birds and consider it probable that the antibodies found in many of the species listed are the result of specific

[1] *Manuscript received July 13, 1942.*

Contribution from the Veterinary Research Station, Lethbridge, Alta., Science Service, Dominion Department of Agriculture.

[2] *Pathologist-in-Charge.*

[3] *Assistant Pathologist.*

infection probably of a mild or unapparent nature. These results indicate that the search for carriers, while admittedly a very large undertaking, has definite justification for its pursuit.

The present report deals with the examination of material during the summer of 1941 for possible carriers of the virus. The procedure used formerly (2, 3) was adopted. Heads and organs of animals to be tested and insects were sent to the laboratory in jars of buffered glycerine solution which were iced for shipment. The brains were removed at the laboratory and placed in buffered glycerine solution. A portion of each brain was ground up with sterile alundum, the remainder being retained for future use if required; 0.2 cc. of each mixture suspended in 0.9% saline solution was injected, under ether anaesthesia, into two guinea pigs by the intracerebral route. In order to conserve guinea pigs, several brains or spleens or other organs from each group of animals to be tested were pooled to form each *lot*, the particulars of which are given below under their lot numbers. The temperature of each guinea pig was taken prior to injection and for at least one week afterwards. Those that showed temperature reactions were destroyed and a suspension of their brains was injected into two or more guinea pigs. All animals that survived for three weeks were given an intracerebral challenge inoculation of 25 M.L.D. (minimum lethal dose) of Western virus.

Lot 1: one engorged female of *Dermacentor andersoni* and 11 males, received from H. S. Seamans, Lethbridge, Alta., on May 14; picked off a horse (normal animal). They were ground up with sterile alundum and 0.2 cc. of the supernatant liquid was injected into two guinea pigs by the intracerebral route. The guinea pigs did not show any abnormal temperature reactions. They were given a challenge inoculation of virus by the same route three weeks later and both died.

Lot 2: brains of two ground squirrels collected by J. H. Brown in a flat west of Irvin, near Medicine Hat, Alta.; received May 26. Half of each brain was ground up and 0.2 cc. of brain suspension was injected into two guinea pigs by the intracerebral route. No abnormal temperature reactions appeared. The guinea pigs were challenged three weeks later, unfortunately with an insufficient dosage, as shown by the controls, and only one died. The remaining half of each brain was injected into two and three guinea pigs, respectively. These animals remained normal and died following challenge inoculation three weeks later.

Lot 3: brains of five ground squirrels, collected at the same place as Lot 2 by J. H. Brown and received the same day. This material was injected in the usual manner into two guinea pigs which, three weeks later, were challenged. They showed no abnormal temperature reactions before being challenged. Only one died, since the challenge dose, as shown by results in controls, was insufficient. To make certain of this, the halves of the five brains, which had been held in the refrigerator, were ground up and the usual injection of each was given to three guinea pigs. One of these 15 guinea pigs showed a temperature of 104.7° F. on the fifth day after injection. It was killed and

a suspension of its brain was injected into four new guinea pigs. The other 14 animals were challenged three weeks later and all died. On the eighth day one of the four guinea pigs had a temperature of 103.6° F. which, however, was reduced to 102.6° on the following day when the animal was killed and a suspension of its brain was injected into two additional guinea pigs. The other three were challenged at the usual time and died. One of the two guinea pigs remained normal; the other had a temperature of 103.8° F. on the fourth day. A suspension of its brain was injected into four guinea pigs all of which remained quite normal. It may therefore be concluded that there was no virus in the brains of the ground squirrels.

Lot 4: four ground squirrel heads, collected at the same place as Lots 2 and 3. Half of each brain was ground up and 0.2 cc. injected in the usual manner into two guinea pigs, the temperatures of which kept within a normal range. They were challenged three weeks later with virus and died following the usual chain of symptoms.

Lot 5: one assassin bug (*Zelus audax*) collected by Dr. R. Salt, Division of Entomology, Lethbridge; received June 21. It was washed in phenolized saline, ground up in 1 cc. of saline, and 0.2 cc. of the suspension was injected into each of two guinea pigs by the intracerebral route. Both animals remained normal as to health and temperature reactions. They were challenged three weeks later with virus and both died.

Lot 6: four ground squirrel brains collected by Mr. J. H. Brown 20 mi. north of Bassano, Alta., and received July 6. The owner of the premises on which these were taken reported the loss of one horse from encephalomyelitis in 1941 and, at the time, had one recovering. There were no human cases on the farm and no other livestock was affected. Ground squirrels were very scarce in this area. The brains were removed at the laboratory and half of each was ground up with saline and pooled. Two guinea pigs were given an intracerebral injection of 0.2 cc. of the suspension. One died two days later as the result of bacterial infection; the other was challenged three weeks after injection and died four days later.

Lot 7: three ground squirrel heads in buffer solution collected from the same place as Lot 6. A suspension of these brains was injected into two guinea pigs. They showed no abnormal temperature reactions and were given a challenge inoculation three weeks after the first injection. Both died five days later with typical symptoms.

Lot 8: one ground squirrel head from the same place as Lot 10. Brain suspension was injected into two guinea pigs and the temperatures were taken as usual. The animals were challenged three weeks later and both died.

Lot 9: livers containing parasites; taken from two ground squirrels which were from the same place as Lot 6. Portions of liver were ground up and the suspension was injected in the usual way into two guinea pigs. Neither animal showed abnormal temperature reactions. They were challenged three weeks later and both died.

Lot 10: four ground squirrel heads collected by Mr. J. H. Brown from a pasture one mile north and one mile west of Countess, Alta. One horse on the premises was recovering from equine encephalomyelitis. There had been no such cases in 1940 and no human cases. The ground squirrel population was reported as normal in number. A suspension of the brains was injected into two guinea pigs without producing any abnormal reaction. They were challenged three weeks later and both died.

Lot 11: two hawk heads from the same place as Lot 10. Brain suspension was injected into guinea pigs by the intracerebral route. Temperatures remained normal. Challenge three weeks later resulted in death.

Lot 12: six ground squirrel heads collected by Mr. J. H. Brown three miles north and one mile west of Countess. The owner reported two cases of equine encephalomyelitis in 1941, five cases in 1938, and two in 1936, none of which were fatal. Of the two horses affected in 1941 one had recovered at the time the report was made. The owner had been affected in 1938 with a disease which he believed he had contracted from the horses. Ground squirrels were reported as having decreased in 1939 and 1940. Brain suspension was injected in the usual manner into two guinea pigs. One died the following day from bacterial infection. The other lived and was challenged three weeks later. It died five days after the challenge inoculation.

Lot 13: head of a burrowing owl, from the same place as Lot 12. This was treated in the usual manner. There were no temperature reactions and both guinea pigs died following challenge inoculations three weeks after injection.

Lot 14: brains of two ground squirrels from the same place as Lot 12. Injection of brain suspension did not give rise to any temperature reactions and both animals died following challenge inoculations three weeks later.

Lot 15: one ground squirrel brain from the same location as Lot 12. A suspension of brain was injected into two guinea pigs by the usual method. One guinea pig did not show any temperature reaction but the other, commencing the following day, had daily temperatures of 105.6°, 101.6°, 105.2°, 105.0°, 104.0°, and 104.0° F. It was killed on the sixth day, the brain was cultured, and the usual brain suspension was injected into two more guinea pigs. These showed no reaction. The original survivor and the second two animals were given a challenge inoculation three weeks after the first injection and all died. The sudden rise of temperature is not typical of virus symptoms but the brain culture showed only one bacterial (coccus) colony. However, the absence of resistance in the challenged animals proves the absence of virus.

Lot 16: brain of jack rabbit, collected by Rocky Mounted Spotted Fever and Sylvatic Plague Survey at the same place as Lot 6 on Aug. 6. A suspension of brain was injected into two guinea pigs. These animals showed no temperature reaction and died following challenge inoculation three weeks later.

Lot 17: this consisted of four ground squirrel heads collected by R.M.S.F. and S.P. Survey on Aug. 7. The brains were suspended in saline and 0.2 cc. was injected into two guinea pigs. One was dead the following day. The other animal did not manifest any unusual temperature reaction. It was challenged three weeks later and died on the fifth day.

Lot 18: six ground squirrel heads collected by R.M.S.F. and S.P. Survey two miles north of Countess on Aug. 7. The owner reported three cases of equine encephalomyelitis in 1941; all of the animals were recovering at the time. One horse was affected in 1936 and one in 1937; both recovered. No humans or other animals were affected. The ground squirrel population had decreased in 1940 and 1941. The owner further reported that some of his chickens had a similar condition in 1938 but as adult fowl do not show symptoms following artificial infection, and paralyses due to other causes are common among them, this is not necessarily important. An injection of 0.2 cc. of pooled brain suspension was given to two guinea pigs. They remained in good health, showing no temperature reaction, and died following challenge inoculation three weeks later.

Lot 19: one ground squirrel head, collected by R.M.S.F. and S.P. Survey on Aug. 7 at the same place as Lot 10. This animal appeared quite ill and was dragging itself around by its forelegs. A suspension of brain was injected into two guinea pigs. One died five days after injection having had a temperature of 105.0° F. from the day after injection. The brain when cultured yielded a heavy growth of Gram negative rods. The other guinea pig ran a high temperature for a few days but the temperature was down to normal within a week. The animal died 17 days after injection. There was no evidence of peritonitis and culture from the brain was sterile. The temperature was probably due to bacterial infection, as when caused by virus it does not appear for several days.

Lot 20: head of a hen which was found dead on Aug. 7 on farm three miles north and four miles east of Duchess, Alta., collected by the R.M.S.F. and S.P. Survey. There were no sick horses on this farm but there was one on a farm two miles south and two miles west. On Aug. 6 a resident of the first farm was admitted to hospital with what was diagnosed as encephalitis. (Blood sample from this man was examined by the authors on Sept. 15 at request of the physician and was positive.) Two guinea pigs were injected with brain suspension. They showed no abnormal reaction and were challenged three weeks after the injection. Both died five days after the challenge inoculation.

Lot 21: two ground squirrel heads collected by R.M.S.F. and S.P. Survey one mile east and one mile north of Gem, Alta., on Aug. 8. The owner reported a registered Percheron stallion affected with encephalomyelitis. He had one case in 1938. He also reported a considerable decrease in the ground squirrel population in 1940 and 1941. He had lost some chickens from no apparent cause. The brains were ground up and 0.2 cc. of suspension was

injected in the usual manner into two guinea pigs. Temperatures of these animals are shown in Table I.

TABLE I

TEMPERATURES OF GUINEA PIGS INJECTED WITH SUSPENSIONS OF GROUND SQUIRREL BRAINS (LOT 21)

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
31	101.8	103.2	101.4	102.4	101.8	104.6	102.4	Killed
32	102.0	104.0	100.8	102.8	102.2	103.3	101.8	101.4

On the fifth day, No. 31 had a temperature of 104.6° F. which was down to 102.4° F. the following day, when the animal was killed. No. 32 showed some temperature rise on the fifth day but was allowed to live. When challenged three weeks later, together with 29 other injected guinea pigs and two controls, it survived the challenging dose of 25 M.L.D. which proved fatal for all the others.

Brain of No. 31 was ground up and injected into two more guinea pigs, the temperatures of which are shown in Table II.

TABLE II

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSION OF GUINEA PIG No. 31

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
84	103.5	102.2	102.0	101.4	102.2	101.4	101.8	102.0
85	102.5	102.7	102.0	101.5	101.2	102.0	101.5	101.6

Nos. 84 and 85 did not show any abnormal temperature following injection. They were challenged with 25 M.L.D. of virus three weeks later and both died.

The original material was injected on Aug. 9 and, owing to the usual period of waiting to challenge, it was not again injected until Sept. 4. At that time the remaining portion of each brain was ground up and injected into two guinea pigs. These are described as Lots 21*A* and *B*. Temperatures of injected guinea pigs are shown in Table III.

TABLE III

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSIONS OF LOTS 21*A* AND *B*

Lot	Guinea pig No.	Days after injection							
		0	1	2	3	4	5	6	7
		Temperature, °F.							
21*A*	124	102.0	102.8	101.2	101.2	102.8	102.8	102.0	101.2
	125	101.2	102.7	103.4	Killed				
21*B*	126	100.0	102.4	101.2	102.0	103.0	103.0	102.0	101.0
	127	102.7	103.0	100.4	101.8	102.0	102.0	101.0	101.6

There was a marked haemorrhage in the brain of No. 125. Cultures showed only a few colonies of bacteria. This brain was ground up and injected into two guinea pigs, temperatures of which are given in Table IV.

TABLE IV

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSION OF GUINEA PIG NO. 125

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
243	102.0	102.0	101.4	101.8	102.4	101.4	101.4	101.8
244	101.0	101.6	101.2	102.3	102.3	102.6	102.0	102.2

Nos. 243 and 244 were challenged with the usual dose of virus three weeks later and both died. Nos. 124, 126, and 127 also died following challenge.

A repeat injection of suspensions of 21*A* and *B* was made six days after that in Table III. Temperatures of these animals are shown in Table V.

TABLE V

TEMPERATURES OF GUINEA PIGS INJECTED WITH SIX-DAY SUSPENSIONS OF LOTS 21*A* AND *B*

Lot	Guinea pig No.	Days after injection							
		0	1	2	3	4	5	6	7
		Temperature, °F.							
21*A*	239	101.0	104.0	101.8	101.0	101.0	101.0	101.0	100.5
	240	101.5	104.4	100.6	101.2	101.8	100.4	101.0	101.6
21*B*	241	100.4	103.0	101.4	101.8	102.4	101.4	101.4	101.8
	242	101.0	101.6	101.2	102.3	102.3	102.6	102.0	102.2

Guinea pigs Nos. 239 to 242 did not show any reaction from the injections. They were challenged at the usual time and all died.

It is believed that the temperature reaction of guinea pig No. 31 taken in conjunction with the immunity that developed in No. 32 may indicate the presence of a trace of virus in the ground squirrel brains. It was not possible, however, to reproduce the immunity in other guinea pigs as was done by Gwatkin and Moore (3). Therefore there is less certainty in the present case that the immunity in guinea pig No. 32 was due to virus in the original ground squirrel brains, but the results suggest this possibility.

Lot 22: six ground squirrel brains collected by the R.M.S.F. and S.P. Survey on Aug. 8 on rangeland four miles south and four miles west of Gem. The owner reported seven cases of the disease in 1941, two of which were fatal. In 1940 he lost one horse and in 1938 he had many cases, 20 of which were fatal. No domestic animals on the premises were ill in 1941 but in 1940 he lost a number of chickens from what appeared to be some brain disease. Ground squirrels were quite scarce. The brains of those collected were ground up and the suspension was injected into two guinea pigs. One had a temperature of 104.7° F. on the fourth day after injection and was killed. The brain yielded a heavy growth of bacteria and was discarded. The other animal did not give a temperature response and died five days after challenge inoculation at the usual time.

Lot 23: one ground squirrel brain collected by the Survey on Aug. 9 on a farm three and one-half miles north and four miles west of Crowfoot. At the time, the owner reported two sick horses, a mare and a colt; both were recovering. In 1938 and 1940 two horses were affected each year. All recovered. In 1941 he lost a number of chickens from no apparent cause. The ground squirrel population was normal in number but was ill in the spring. In 1938, also, the owner had noticed that the ground squirrels were ill, dragging themselves along by their forelegs and appearing to stagger. Guinea pigs were injected as usual with brain suspension and remained normal. They died following challenge inoculation three weeks later.

Lot 24: five ground squirrel heads from same place as Lot 23. Guinea pigs injected in the usual manner died from bacterial infection.

Lot 25: two ground squirrel heads from same place as Lot 23. Injected guinea pigs died from bacterial infection.

Lot 26: two ground squirrel heads from same place as Lot 23. Injected guinea pigs both ran high temperatures but as the squirrel heads were in the same shipment as Lots 24 and 25, it was believed that the reaction was due to bacterial infection. This was confirmed by challenge inoculation three weeks later, at which time both guinea pigs died.

Lot 27: portion of liver of ground squirrel from same place as Lot 23. Intracerebral injection in guinea pigs resulted in death of these animals from bacterial infection.

Lots 28 and 29: each lot consisted of one cat brain from Rose Lynn and Hanna, Alta. Both lots were in bad condition and injected guinea pigs died from bacterial infection.

Lot 30: one chicken head collected by the Survey on Aug. 14 at a farm near Watts. The owner had lost 17 chickens and some pigs in 1941 and some chickens in 1940. In 1938, three colts and one 12-yr. old horse suffered from encephalomyelitis. In 1940 the ground squirrels became scabby and a number died. Injected guinea pigs showed no temperature reactions and died following challenge inoculation three weeks later.

Lot 31: two pig brains from the same place as Lot 30. These pigs were three weeks old. One had died and the other was quite ill. Examination disclosed a spotted spleen and haemorrhagic intestines. "They appeared to be suffering from haemorrhagic septicaemia". A suspension of this material was injected into guinea pigs, which remained well and were challenged three weeks later, dying five days after the challenge inoculation.

Lot 32: one head of domestic chicken collected by the Survey at a farm near Hanna. The bird was paralysed and had been ill four days. Twelve had died in the same manner. The owner had also lost about 45 spring turkeys. Twenty chickens had been lost in 1938. No horses were sick. The ground squirrels were said to be mangy. A suspension of this brain was injected into two guinea pigs, one of which was dead the following day from bacterial infection. The other was challenged three weeks later and was dead on the fifth day after the challenge inoculation.

Lot 33: brains of three ground squirrels collected by the Survey on a farm near Rose Lynn adjacent to a pasture where a filly developed encephalomyelitis. (No ground squirrels were seen in this pasture.) Before the horse took sick a pig became paralysed and died. Ground squirrels were on the decrease in 1940 and 1941. A suspension of the brains was injected into two guinea pigs. One died the following day from bacterial infection. The other showed some temperature reaction for a few days and then returned to normal. It died following a challenge inoculation three weeks later.

Lot 34: six ground squirrel heads collected by the Survey near Rose Lynn on Aug. 17. The owner reported having lost one horse in 1941 from encephalomyelitis and 25 chickens from range paralysis. No other domestic animals were affected. The ground squirrel population was apparently normal in number. In 1938 he lost three colts. A suspension of these brains was injected in the usual manner into two guinea pigs. One was dead the following day and the brain yielded a heavy growth of bacteria. The other survived and, three weeks later, was given a challenge inoculation to which it succumbed.

Lot 35: five ground squirrel heads from the same shipment as Lot 34. Two heads were discarded owing to injury from shot. The remaining three were ground up and injected into two guinea pigs but both animals were dead the following day from bacterial infection.

Lot 36: four ground squirrel heads collected by the Survey, Aug. 29, on a farm near Enchant, Alta. Two horses had been affected with encephalomyelitis in 1941 and one of them died. The brains were ground up and injected in the usual manner into two guinea pigs. These animals showed no abnormal reaction and died following challenge inoculation three weeks later.

Lot 37: one jack rabbit head. This animal was collected by the Survey at the same place as Lot 36. Guinea pigs were injected with the brain and challenged three weeks later, following which both died.

Lot 38: seven ground squirrel heads from a farm near Enchant collected by the Survey on Aug. 29. Two horses came down with encephalomyelitis in the pasture in which these were collected. On a farm adjacent to this a woman died of encephalitis. Ground squirrels were very plentiful. Three heads in this lot had to be discarded on account of injury which made them unsuitable for injection. The others were ground up and injected into two guinea pigs. These animals did not show any temperature reaction and died following challenge inoculation of virus three weeks later.

Lot 39: five assassin bugs (*Sinea diadema*) collected by H. L. Seamans in the Swift Current–Vanguard district in Saskatchewan. They were washed in phenolized saline, ground up, and injected into two guinea pigs. One of these animals was dead the following day and was replaced with another, which also died. The survivor had a temperature of 105° F. on the second day after injection but returned to normal. It died following challenge three weeks later. Loss of the two guinea pigs was due apparently to some toxic property.

This material was held for a time to see if the toxic quality would disappear. The suspension was then filtered through a Berkefeld candle (V). Growth occurred in the filtrate so it was refiltered, after which it proved sterile. Two guinea pigs were injected by the intracerebral route with 0.2 cc. each. Both were dead the following morning.

Only one of the five guinea pigs injected with this lot survived but, as shown above, it did not show any evidence of the presence of virus.

Lot 41: five mosquitoes collected at the Klinck farm, Yellowgrass, Sask., on Sept. 4. They were washed in phenolized saline, ground up, and injected into two guinea pigs. These animals showed no temperature response and died following inoculation of virus three weeks later. Lots 41 to 76 were collected in Saskatchewan in connection with the investigation concerning encephalitis.

Lot 42: thirty mosquitoes collected at Weyburn, Sask. and preserved in buffer solution. They were washed in phenolized saline, ground up, and 0.2 cc. of suspension was injected into two guinea pigs by the intracerebral route. The injected guinea pigs showed no temperature response and died following the usual challenge inoculation of 25 M.L.D. of virus three weeks later.

NOTE: *Lot 40 was human spinal fluid; results not reported here.*

Lot 43: thirty-three mosquitoes collected at Weyburn, Sask. They were handled as Lot 42. Guinea pigs remained well and died following the challenge inoculation.

Lot 44: thirty-five mosquitoes collected at Weyburn. The injected guinea pigs remained well and died following challenge three weeks later.

Lot 45: thirty mosquitoes collected at Weyburn. Injected guinea pigs remained well and died following the challenge inoculation three weeks later.

Lot 46: spleen of hawk collected 10 mi. west of Weyburn. A portion of the spleen was ground up with alundum and injected by the intracerebral route into two guinea pigs. One of these was dead the following day. The survivor showed no temperature reaction and died following the challenge inoculation three weeks later.

Lot 47: four ground squirrel heads collected in the vicinity of Ceylon on Sept. 1. They were treated in the usual manner. The two injected guinea pigs showed no temperature reaction and died following the usual challenge inoculation.

Lot 48: two hawk heads collected 10 mi. west of Weyburn on Sept. 1. Suspension of brains was injected into two guinea pigs. No temperature reactions occurred and the guinea pigs died following the challenge inoculation three weeks later.

Lot 49: two duck heads collected at Kleinsaucer Lake on Sept. 1. Autopsy of these animals suggested the occurrence of Western duck sickness. Moreover, ducks were sick on this lake which is very shallow and of the type in which duck sickness occurs; not a great number of dead ducks were seen however—perhaps 25. Suspensions of the brains were injected in the usual manner into guinea pigs, which showed no temperature reactions. They died following challenge inoculation three weeks later.

Lot 50: two ground squirrel heads collected at the Klinck farm near Yellowgrass. Injected guinea pigs showed no reaction to the injection of brain suspension and died following the usual challenge inoculation three weeks later.

Lot 51: two ground squirrel spleens from the Klinck farm, collected Aug. 31. These were ground up and injected in the usual manner. The injected guinea pigs showed no reaction and died following the challenge inoculation three weeks later.

Lot 52: brain of hawk found dead at Kleinsaucer Lake. Injected guinea pigs remained well and died following challenge inoculation three weeks later.

Lot 53: six ground squirrel heads collected five miles northeast of Weyburn on Sept. 2. A suspension of these brains was injected into two guinea pigs in the usual manner. These animals gave temperature reactions which are shown in Table VI.

No. 156 was killed on the fourth day after injection when the temperature was 105.6° F. The brain was removed, examined microscopically, and cultured. Many gram negative rods were seen on direct smears and cultures

TABLE VI

TEMPERATURES OF GUINEA PIGS INJECTED WITH SUSPENSION OF GROUND SQUIRREL BRAINS

Guinea pig No.	Days after injection									
	0	1	2	3	4	5	6	7	8	
	Temperature, °F.									
155	100.2	101.6	101.8	101.2	102.0	103.5	103.0	103.2	104.2	Killed
156	100.3	102.0	103.0	105.8	105.6	Killed				

yielded a heavy growth of the same organism. No. 155 was killed on the eighth day when the temperature was 104.2° F. The brain yielded only one colony of bacteria and was injected into two guinea pigs three days after removal. The temperatures of these animals are given in Table VII.

TABLE VII

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSIONS OF No. 155

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
270	100.0	101.4	101.6	101.2	101.2	101.0	101.6	101.6
271	101.2	102.0	102.6	102.3	102.0	101.8	102.8	102.0

Nos. 270 and 271 did not show any abnormal temperature reaction and remained in good health. They died following challenge inoculation three weeks later so it may be assumed that the original temperature reactions were due to bacterial infection.

Lot 54: five ground squirrel heads collected five miles northeast of Weyburn on Sept. 2. The brains were ground up, pooled, and injected into two guinea pigs. These animals showed no temperature reaction and died following a challenge inoculation three weeks later.

Lot 55: four spleens from ground squirrels (Lot 54). Injected guinea pigs showed no reaction and died following challenge inoculation three weeks later.

Lot 56: three ground squirrel spleens from the same place as Lot 54. The injected guinea pigs gave no reaction following injections of spleen and died after a challenge inoculation three weeks later.

Lot 57: three hawk heads, collected on highway No. 13 near Hume, Sask. Guinea pigs injected with brain suspension remained normal and died following challenge inoculation three weeks later.

Lot 58: four hawk spleens. Guinea pigs injected with the material showed no temperature reaction. They died following challenge inoculation three weeks later.

Lot 59: two hawk heads and one ground squirrel head, collected on highway No. 13, near Hume, on Sept. 3. There was no reaction following injection of this material and the guinea pigs died after a challenge inoculation three weeks later.

Lot 60: four ground squirrel heads, collected on Sept. 4, three miles southwest of Hume. The injected guinea pigs did not show any reaction and died following the subsequent challenge inoculation.

Lot 61: four ground squirrel heads, collected Sept. 4, at a point three miles southwest of Hume. One head had been injured and was discarded. The other brains were ground up and injected into two guinea pigs. One of these animals showed a slight temperature rise on the eighth day after injection and was killed and the brain removed. Temperatures are shown in Table VIII.

TABLE VIII

TEMPERATURES OF GUINEA PIGS INJECTED WITH GROUND SQUIRREL HEADS (LOT 61)

Guinea pig No.	Days after injection									
	0	1	2	3	4	5	6	7	8	
	Temperature, °F.									
171	99.0	102.0	101.8	101.4	101.4	101.0	101.8	101.8	101.0	
172	100.4	103.0	103.0	102.6	102.0	102.0	102.0	103.5	103.6	Killed

No. 172 was killed on the eighth day after injection as it was showing some increase in temperature. The brain was ground up and injected into another two guinea pigs, temperatures of which are shown in Table IX.

TABLE IX

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSION OF GUINEA PIG NO. 172

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
272	100.0	101.6	103.4	102.5	102.2	102.4	102.0	101.6
273	101.5	101.8	102.0	101.6	102.2	101.6	101.8	102.4

Guinea pigs Nos. 272 and 273 showed no reaction and died following challenge injection three weeks after the first injection. No. 171 was also challenged three weeks after the first injection and died with the usual train of symptoms.

Lot 62: six ground squirrel spleens collected Sept. 4 at a point three miles southwest of Hume. A portion of each spleen was ground up together and 0.2 cc. of suspension injected into two guinea pigs. Temperatures of these animals are shown in Table X.

TABLE X

TEMPERATURES OF GUINEA PIGS INJECTED WITH SUSPENSIONS OF SPLEENS (LOT 62)

Guinea pig No.	Days after injection									
	0	1	2	3	4	5	6	7	8	
	Temperature, °F.									
173	101.8	101.4	100.8	101.0	101.4	101.6	101.8	103.6	103.8	Killed
174	100.4	101.8	101.8	100.6	101.2	101.2	103.8	103.7	99.0	Killed

Both guinea pigs showed a temperature rise on the seventh day after injection. Both animals were killed on the eighth day, the brains removed, and cultured. At this time No. 174 was ill and its temperature was going down. There was evidence of peritonitis in No. 174 but not in No. 173. Brain cultures of both were negative. Blood of No. 173 was collected for neutralization test, the result of which was questionable. Peritoneal cultures of No. 173 were negative but No. 174 yielded a heavy growth of bacteria.

The brain of No. 173 was ground up and injected into guinea pigs, Nos. 274 and 275, two days after removal. Temperatures of these animals are given in Table XI.

TABLE XI

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSION OF GUINEA PIG NO. 173

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
274	100.6	101.5	102.6	102.0	101.7	101.8	102.0	101.4
275	101.0	101.2	103.4	101.8	102.7	102.8	102.6	102.4

Nos. 274 and 275 remained well and died following the usual challenge inoculation. Nothing was done with brain of No. 174 as death of this animal was due to peritonitis.

Lot 63: thirty stable flies (*Stomoxys* sp.) collected near Trossachs on the highway on Sept. 1. They were washed, ground up, and injected into guinea pigs in the usual manner. One guinea pig showed a marked temperature rise on the second day after injection (105.8° F.) the other, a temperature of 103.8° F. on the same day. Both rapidly returned to normal. Because of its early appearance the reaction was ascribed to bacterial infection, or toxic property of the flies, and this was confirmed by challenge inoculation of these animals three weeks later, following which both animals died.

Lot 64: nine specimens of *Chrysops* sp. and one of *Tabanus* sp. collected Sept. 1 on the highway near Trossachs. These were washed, ground up, and injected into two guinea pigs, which showed no reaction. They died following subsequent challenge inoculation.

Lot 65: four rat heads (*Mus decumanus*) collected at Weyburn waste disposal ground. They were injected in the usual way into two guinea pigs, which did not show any disturbance and which died following the usual challenge three weeks later.

Lot 66: four rat heads (*M. decumanus*) from same source as Lot 65. Guinea pigs were injected in the usual manner. Temperatures of these animals are shown in Table XII.

TABLE XII

TEMPERATURES OF GUINEA PIGS INJECTED WITH RAT BRAINS (LOT 66)

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
183	100.8	102.0	105.0	104.0	104.4	102.4	103.0	102.2
184	102.0	102.2	104.6	102.2	101.0	101.8	102.4	102.2

No. 183 died 21 days after injection. Death was due to peritonitis and the brain was discarded. No. 184 was challenged about three weeks after the first injection and died five days later.

Lot 67: one black rat head (*Mus rattus?*) collected at the same place and time as Lots 65 and 66. Both guinea pigs injected with brain suspension remained well, showed no temperature reaction and died following subsequent challenge inoculation.

Lot 68: one brain of domestic pig which died near Weyburn. Autopsy was conducted at the request of Dr. A. J. Andries but, owing to decomposition of the carcass, was not satisfactory. The brain was collected and a suspension prepared and injected into guinea pigs in the usual manner. The brain must have been cleaner than the carcass for the injected guinea pigs did not show any reaction. They died following the usual challenge inoculation.

Lot 69: six rat spleens removed from some of the rats collected at Weyburn. They were ground up and injected in the usual manner into two guinea pigs, which remained well and died following challenge inoculation.

Lot 70: fifteen black crickets collected near Lang around a house where a patient had encephalitis. Two guinea pigs injected with suspension were dead the following morning as a result of infection.

Lot 71: twelve black and three light coloured crickets collected in the same place as Lot 70. One of the injected guinea pigs survived the injection but showed an immediate rise of temperature. This animal died following subsequent challenge inoculation.

Lot 72: twelve grasshoppers collected between the Klinck farm and Weyburn. They were washed in phenolized saline, as was done with all the insects, ground up with sterile alundum, and the usual injection made into two guinea pigs. These animals showed no abnormal temperature reaction and died following injection of the challenge dose three weeks later.

Lot 73: twelve grasshoppers from the same place as Lot 72. Guinea pigs were injected in the same manner but both died the following day, owing to bacterial infection.

Lot 74: forty grasshoppers, from the same source as Lot 73, were ground up with alundum to make a saline suspension. This was filtered through paper pulp and a Berkefeld candle (V) and 0.2 cc. was injected into each of two guinea pigs. These animals did not show any abnormal reaction and both died following the usual challenge inoculation.

Lot 75: this consisted of suspensions of Lots 70 and 71, pooled and filtered through paper pulp and a Berkefeld candle (V). This was injected into two guinea pigs by the intracerebral route. One of these animals, No. 266, died 11 days after injection. The brain showed the presence of gram positive rods which did not grow on aerobic culture. The other animal, No. 267, was given a challenge inoculation three weeks later and died with the usual train of symptoms.

Lot 76: this consisted of filtrate of Lot 39 and has been reported under that number.

Lot 77: three pheasant heads received Sept. 13 from Mr. G. Allen Mail of Kamloops. They were shot less than two miles from a farm where two horses were down with encephalomyelitis the previous month. Two guinea pigs were injected in the usual manner with the suspension and showed no reaction. They both died following challenge inoculation.

Lot 78: heads of four crows from Mr. G. Allen Mail, Kamloops, shot on the laboratory property where one horse died in 1940 of encephalomyelitis. Two guinea pigs were injected with brain suspension. They remained well and died following the subsequent challenge inoculation three weeks later.

Discussion

Lot 21, consisting of two ground squirrel brains, was the only group to show any evidence suggestive of the presence of virus. Both guinea pigs injected with a suspension of these brains gave a temperature reaction. One was killed and a suspension of its brain was injected into other guinea pigs, with negative results. The surviving guinea pig of the original pair resisted a subsequent challenge inoculation, which proved fatal for the two controls and 29 other guinea pigs injected at the same time.

The temperature reactions and resistance to challenge of the surviving guinea pig suggests the presence of a trace of virus in the ground squirrel brains. Unfortunately, by the time the challenge results were known the original material had been held for 26 days, which would result in an even lower concentration of virus, if such were present, than at the time of the first injection.

Acknowledgments

Lots 2 to 4 and 6 to 38 were collected by Mr. J. H. Brown, Officer-in-Charge, and Messrs. G. D. Roy, J. L. MacLeod, and J. W. Duggan of the Rocky Mountain Spotted Fever and Sylvatic Plague Survey of Alberta, to whom the authors are grateful for these specimens and the information gathered at the places of collection. Lots 1 and 39 were received through the kindness of Mr. H. L. Seamans, Entomologist-in-Charge, and Lot 5 through that of Dr. R. Salt, Division of Entomology, Science Service, Lethbridge. Lots 41 to 74 were collected by one of the authors (R.G.) with the assistance of Mr. J. Hockley of the Department of Health of Saskatchewan. The authors are greatly indebted to Dr. R. O. Davison, Deputy Minister of Health of that province for generously placing the services of members of his staff and facilities of his department at their disposal. They would also like to acknowledge the co-operation of Dr. R. J. Gibbons, Assistant Chief, Laboratory of Hygiene, Department of Pensions and National Health, in connection with this group. Lots 77 and 78 were kindly sent by Mr. G. Allen Mail, Livestock Insect Laboratory, Kamloops, B.C. They gratefully acknowledge the advice and support of Dr. E. A. Watson, Dominion Animal Pathologist, in connection with this work.

References

1. Cox, H. R., Jellison, W. L., and Hughes, L. E. U.S. Pub. Health Repts. 56 : 1905–1906. 1941.
2. Gwa[t]kin, R. Can. J. Comp. Med. 5(4) : 113-116. 1941.
3. Gwatkin, R. and Moore, T. Can. J. Comp. Med. 4(3) : 78-82. 1940.
4. Hammon, W. McD., Gray, J. A., Jr., Evans, F. C., Izumi, E. M., and Lundy, H. W. Science (n.s.), 94(2439) : 305-307. 1941.
5. Hammon, W. McD., Reeves, W. C., Brookman, B., Izumi, E. M., and Gjullin C. M. Science (n.s.), 94(2440) : 328-330. 1941.

SOME RECENT BIRD RECORDS FROM CANADA'S EASTERN ARCTIC[1]

By T. M. Shortt[2] and H. S. Peters[3]

Abstract

The list includes 82 species of birds from Canada's eastern Arctic. Opportunity for field observation was afforded the authors, separately, during two voyages of the *Nascopie* (1938 and 1939). Annotations concern numbers, dates of occurrences, habits, and distribution. Notable range extensions are recorded. Taxonomic notes are based on comparisons of a collection secured in 1938 and on additional unreported material recently acquired from the region by the Division of Birds, Royal Ontario Museum of Zoology.

Since 1933 the R.M.S. *Nascopie* has carried the Canadian Government Arctic Patrol on its annual visit to the eastern Arctic. The personnel is composed chiefly of administrators, medical officers, and scientists whose purposes are to supervise the health and general welfare of the natives and to make scientific investigation of the region.

The following annotated list of birds is based primarily on the authors' observations made while attached to the Government party on board the *Nascopie* in 1938 (T.M.S.) and 1939 (H.S.P.). On the 1938 trip, 173 bird specimens of 44 species were preserved. In addition, information derived from recent collections which have been made in the region and which have been acquired by the Royal Ontario Museum of Zoology have been included. These collections consist of: 109 skins from Somerset Island, collected by L. A. Learmonth of the Hudson's Bay Company in 1938–40; 59 birds taken at Lake Harbour, Baffin Island, by Rev. C. L. W. Bailey in the years 1930–32; and a few specimens secured by Rev. D. B. Marsh at Eskimo Point, N.W.T., approximately 160 mi. north of Churchill, Man.

The list is not intended as a catalogue of the birds of the eastern Arctic. It places on record a number of range extensions and new occurrences. Records for the two years are given separately in the text.

For the sake of brevity, dates have been omitted except where essential. However, the itinerary of the *Nascopie's* cruises for 1938 and 1939 has been included (Table I and Fig. 1) as an index to time and place.

Annotated List

Gavia immer (Brünnich). Common Loon.—*1938:* Two adults were noted on a small lake near Glasgow Falls, Lake Harbour, southern Baffin Island on July 20 and one individual was observed at Sugluk West, Que. At Pangnirtung, Baffin Island, skins of this species were seen in mats made by Eskimos. Information to the effect that these birds had been taken locally was secured from the Royal Canadian Mounted Police. *1939:* Five were seen a few miles east of Greenly Island in Belle Isle Strait on July 11.

[1] *Manuscript received May 13, 1942.*

Contribution from the Royal Ontario Museum of Zoology, Toronto, Ont. and the Fish and Wildlife Service, United States Department of the Interior, Charleston, S.C.

[2] *Artist-Associate, Division of Birds, Royal Ontario Museum of Zoology.*

[3] *Atlantic Flyway Biologist, Fish and Wildlife Service, U.S. Department of the Interior.*

TABLE I

ITINERARY OF THE R.M.S. *Nascopie* IN 1938 AND 1939

Port of call	1938		1939	
	Arrived	Departed	Arrived	Departed
Montreal, Que.	—	July 9	—	July 8
Hebron, Labrador	July 15	16	July 17	18
Port Burwell, N.W.T.	17	18	19	20
Lake Harbour, N.W.T.	20	21	22	23
Wakeham Bay, Que.	21	22	24	25
Sugluk West, Que.	22	23	25	26
Cape Dorset, N.W.T.	24	26	27	28
Wolstenholme, Que.	26	27	28	29
Southampton I., N.W.T.	28	29	30	31
Cape Smith, N.W.T.	30	31	Aug. 1	Aug. 1
Port Harrison, Que.	Aug. 2	Aug. 4	2	4
Churchill, Man.	6	8	6	8
Chesterfield, N.W.T.	10	12	10	12
Wolstenholme, Que.	13	14	13	14
Lake Harbour, N.W.T.	16	17	15	15
Port Burwell, N.W.T.	18	19	16	16
Thule, Greenland	24	25	—	—
Craig Harbour, N.W.T.	26	26	22	24
Arctic Bay, N.W.T.	28	29	Sept. 3	Sept. 4
Fort Ross, N.W.T.	31	Sept. 1	Aug. 29	Aug. 31
Pond Inlet, N.W.T.	Sept. 3	4	Sept. 5	Sept. 6
Clyde River, N.W.T.	5	6	7	8
Pangnirtung, N.W.T.	9	12	12	15
Hebron, Labrador	14	15	18	19
Halifax, N.S.	19	Voyage ends	23	Voyage ends

Six specimens of the common loon were received in the collections made by Bailey. These had been taken at Lake Harbour and Saddleback Island, Baffin Island, at various dates in June and July, 1930 and 1932. The fact that these birds are not sexed, together with a lack of sufficient comparative material, makes it unwise to venture an opinion on their subspecific status. They are, however, small in size, as the average measurements show: wing, 356 mm., (348–371); culmen, 79.4 mm. (75–82.5); depth of bill, 24.6 mm. (23–26.5); tarsus, 87.4 mm. (86.5–89); outer toe, 110 mm. (108–113).

Gavia adamsi (Gray). YELLOW-BILLED LOON.—*1938:* Two adults of this species were seen on a small lake near Fort Ross, Somerset Island. These were readily attracted to a waving handkerchief and the female was taken. An immature loon of large size, presumably of this species, was observed on the sea at the east end of Bellot Strait on the same day.

Learmonth secured this species on the southeast coast of Somerset Island on June 30, 1938.

These records, coupled with one from the Melville Peninsula and reports from Foxe Basin (8), form a considerable north-eastward extension in the known range of *G. adamsi* and indicate that Ross's early record (3) for the Boothia Peninsula was within the normal range of the species.

Gavia arctica (Linnaeus). ARCTIC LOON.—*1938:* Five Arctic loons were seen near the post at Coral Harbour, Southampton Island, and an adult female was collected. Single birds were seen at Chesterfield, N.W.T., Arctic Bay, and Pond Inlet, Baffin Island. *1939:* One was observed at Churchill and one at Chesterfield.

Bailey collected two specimens, one at Lake Harbour at the end of May, 1932, and the other on Saddleback Island on June 17, 1930. He also secured an egg from a nest which was situated on an island off Big Island on June 18, 1930. This nest was located in the midst of a colony of common eider duck.

Gavia stellata (Pontoppidan). RED-THROATED LOON.—*1938:* This loon was observed at more posts and in greater numbers than any of the preceding. These observations were: at Chesterfield, N.W.T., two adults; Cape Dorset, Baffin Island, one adult; Fort Ross, Somerset Island, two adults; River Clyde, Baffin Island, one adult, two young; Pond Inlet, Baffin Island, two adults, two young; Churchill, Man., one adult; Coral Harbour, Southamp-

FIG. 1.

ton Island, one adult. A male was collected at Pond Inlet. It was still in full summer plumage. *1939:*. The most common loon seen on the trip. One seen at Lake Harbour, Baffin Island; about 20 at Southampton Island; two at Port Harrison, Que.; one at Chesterfield, N.W.T.; one in Kennedy Harbour, Fort Ross, Somerset Island; and one at Pond Inlet, Baffin Island.

Bailey sent four breeding specimens from Lake Harbour. These had been taken in July, 1931, and in June 1932.

Learmonth collected five specimens on Somerset Island. These were breeding adults taken at Fort Ross and Port Leopold during June and July, 1938, June, 1939, and July, 1940.

Podilymbus podiceps (Linnaeus). PIED-BILLED GREBE.—Bailey secured a specimen which had been taken by a native in November, 1932, at Fair Ness, Baffin Island. This record has been published by Snyder (4).

Puffinus gravis (O'Reilly). GREATER SHEARWATER.—*1938:* Seen in some numbers off the Labrador coast near Makkovik on July 14. *1939:* First seen on July 11 in the western part of Belle Isle Strait. Several seen off Cartwright, Labrador, on July 12. On the return trip they were first met off Hamilton Inlet, Labrador, on September 20.

Fulmarus glacialis (Linnaeus). NORTHERN FULMAR.—*1938:* The most common bird at sea. Observed north to Lancaster Sound and adjacent latitude in Baffin Bay. *1939:* First encountered in the vicinity of Cape Chidley, where they were common, and in Gray Straits on July 19. None seen westward in Hudson Strait or in Hudson Bay. Abundant around Port Burwell and the Button Islands on August 16. Common in Davis Strait, Baffin Bay, Jones Sound, and even in Craig Harbour, Ellesmere Island. Fairly common in Lancaster Sound, Prince Regent Inlet, and at Fort Ross and the eastern end of Bellot Strait. Some were present in Admiralty Inlet and Adams Sound near Arctic Bay. A few in Pond Inlet and Cumberland Sound. Common all along the Labrador coast at least as far south as Cartwright. This was the most common bird at sea in the inlets and sounds north of Hudson Strait.

Learmonth secured a pair in July, 1939, and eight on May 4, 1940, at Port Leopold, Somerset Island, all in the light phase.

Cygnus columbianus (Ord). WHISTLING SWAN.—*1939:* Three observed flying over the shoreline of Southampton Island.

Marsh collected a male on May 30, 1937, at Eskimo Point.

Branta canadensis (Linnaeus). CANADA GOOSE.—*1938:* Seen only at Lake Harbour, Baffin Island, where two immatures were observed and one of them was collected on August 17. This specimen is referable to *B. canadensis canadensis*. *1939:* At Lake Harbour on August 15, fresh tracks and droppings were found on the sandy beach of a lake a few miles inland from the post.

Bailey found a nest containing four eggs at Ashe Inlet, Big Island, on June 17, 1930. He collected and preserved one egg.

A female was taken by Marsh on May 20, 1937, at Eskimo Point. This specimen appears to be of the form *leucopareia* (Brandt) as defined by Taverner (7). Its measurements are: culmen, 43 mm.; depth of bill, 19 mm.; tarsus, 69 mm.; middle toe and claw, 71 mm.; wing, 385 mm. The white cheek patches are divided by a broad black line along the throat and chin.

Branta bernicla Linnaeus. COMMON BRANT.—*1938:* A flock of about 10 birds of this species was observed in Lancaster Sound off Devon Island on August 27. *1939:* A flock of about 15 birds, seen flying eastward in Lancaster Sound on August 26, was probably of this species but was too far away for positive identification.

Learmonth secured a male at Port Leopold on June 21, 1939. He states that this species is "quite rare" on Somerset Island.

Chen hyperborea (Pallas). SNOW GOOSE.—*1939:* On September 6, at Pond Inlet, near the mouth of the Salmon River, numerous signs of this species were seen although the birds were reported to have been observed last on August 31.

Learmonth secured a female and a downy young on July 18, 1938, on the southeast coast of Somerset Island. The adult bird, on the basis of size, is referable to *C. hyperborea atlantica* Kennard, the greater snow goose. Its measurements agree well with those given by Kennard (2) for *atlantica* and are: wing, 459 mm.; exposed culmen, 64 mm.; depth of culmen, 36 mm.; middle toe and claw, 84 mm.; tarsus, 89 mm. In addition the bird has the heavy, robust build mentioned by Kennard. It is doubtful, however, if this bird is representative of the Somerset Island snow goose population.

Anas platyrhynchos Linnaeus. MALLARD DUCK.—*1938:* Several small flocks observed at Churchill, Man., on August 6. A flock of four, flying northward, was observed in Hudson Bay about 100 miles south of Chesterfield, probably off Tavani.

Anas rubripes Brewster. BLACK DUCK.—*1938:* Noted only at Churchill, where five birds were seen. *1939:* One seen on a small pond in the townsite at Churchill on August 7.

Dafila acuta (Linnaeus). PINTAIL.—*1938:* A large flock seen at Churchill over the marsh along the river and two birds observed in flight northward about 50 miles south of Chesterfield, N.W.T., on August 9. *1939:* Six observed in a small pond in the townsite at Churchill. Reported to be nesting around Chesterfield, but none seen during our stay.

A male was taken by Marsh at Eskimo Point on June 30, 1938.

Nettion carolinense (Gmelin). GREEN-WINGED TEAL.—*1938:* Two green-winged teal noted on August 6 at Churchill. *1939:* Two seen in the townsite slough at Churchill, August 7.

Clangula hyemalis (Linnaeus). OLD-SQUAW.—*1938*: The most frequently observed duck throughout most of the trip, especially in the waters about the Arctic Islands. Flocks of 20 to 100 were seen at Pond Inlet; River Clyde, Baffin Island; and Fort Ross, Somerset Island. A nest with eight eggs was found near the post at Coral Harbour, Southampton Island. Two downy young were collected from a flock of eight with parent at Chesterfield, N.W.T. *1939:* At Southampton Island two adults were seen with about 20 young which had not attained the power of flight. Two adults and about 20 young were also seen at Churchill. Several adults and about 40 young at Chesterfield. An adult was seen in an arm of Hazard Bay at Fort Ross. A raft of about 70 was found at the mouth of the Salmon River at Pond Inlet; three of these were young and still unable to fly.

Learmonth secured two adults at Port Leopold on June 20, 1940.

Histrionicus histrionicus (Linnaeus). HARLEQUIN DUCK.—*1939:* A pair was seen flying over the harbour at Lake Harbour, on July 22.

Somateria mollissima Linnaeus. COMMON EIDER DUCK.—*1938:* Next to the old-squaw, this was the most conspicuous duck. Three flocks of about 50 each were seen at River Clyde, Baffin Island. Individuals or small flocks were seen at Pond Inlet, Arctic Bay, Southampton Island, and Chesterfield. An adult female was taken from a flock of 12 at Chesterfield. This specimen is characteristic of the recently described race, *sedentaria* Snyder (5). A few small flocks were noted in Davis Strait. *1939:* This species was seen as follows: eleven flying in Gray Straits on July 19; 10 flying over Lake Harbour on July 22; several males seen at Sugluk West on July 25; one at Cape Dorset; a flock of 20 near Cape Wolstenholme on July 28; and about 20 near Southampton Island.

Bailey collected breeding birds at Lake Harbour, Saddleback Island, and on an island off Crooks Inlet, southern Baffin Island. He also secured eggs from a colony on Big Island which, according to his report, contained "hundreds of nests".

Learmonth took male specimens at Cresswell Bay on June 21, 1938, and at Port Leopold on July 12, 1939, and June, 1940. These birds are referable to *borealis* Brehm and probably represent the most westerly occurrence of this form.

Marsh collected a female at Eskimo Point on July 5, 1937.

Somateria spectabilis (Linnaeus). KING EIDER.—*1938:* This species was noted only sparingly. Three were noted off Cape Dorset, two were seen at Pangnirtung; a male was collected at Craig Harbour, Ellesmere Island. *1939:* Positively identified only at Southampton Island, where two were seen.

Bailey took one on July 11, 1932, at Lake Harbour.

Learmonth collected females at Cresswell Bay on June 15 and July 1, 1938, and a series of both sexes at Port Leopold in 1940. The earliest date among these is May 11.

Oidemia americana (Swainson). AMERICAN SCOTER.—*1938:* A pair of scoters, the male of which was all black, was seen flying southeast in Hudson Bay off Port Harrison on August 2.

Mergus serrator Linnaeus. RED-BREASTED MERGANSER.—Bailey secured an adult male of this species at Lake Harbour, Baffin Island, in 1932. The bird, which shows a few traces of the eclipse plumage, was presumably taken by natives in the fall.

Buteo lagopus (Brünnich). COMMON ROUGH-LEGGED HAWK.—*1938:* Pairs were noted at Hebron, Labrador; Lake Harbour, Baffin Island; Cape Smith, N.W.T.; Wakeham Bay and Cape Wolstenholme, Que. They apparently were nesting on the cliffs at each of these localities. Adult specimens were secured at Cape Smith on July 30, and at Cape Wolstenholme, August 14. *1939:* One observed a short distance inland from Port Harrison, probably nesting nearby.

Circus hudsonius (Linnaeus). MARSH HAWK.—*1938:* An adult male was seen on August 6 at Churchill, Man. *1939:* One in brown plumage was seen several miles inland from Churchill, August 7.

Falco rusticolus Linnaeus. GYRFALCON.—*1938:* Definitely identified only at Cape York, Greenland, where a white individual was observed flying over the sea near the coast. A freshly shed unsoiled feather which was picked up at Pangnirtung, Baffin Island, has been positively identified as white gyrfalcon. Dark falcons were seen at long range near Thule, Greenland, and near Hebron, Labrador. These were not identified, but the former was most likely a gyrfalcon. *1939:* A black adult was seen soaring over a cliff several miles inland from Lake Harbour on August 15.

Bailey took one at Lake Harbour on August 11, 1930.

Learmonth collected one at Bellot Strait on November 5, 1938.

Both of these birds are typically white specimens of *F. candicans* Gmelin.

Falco peregrinus Tunstall. PEREGRINE FALCON.—*1938:* Individuals were seen at Sugluk West, Que., and at Churchill, Man. A falcon observed at River Clyde, Baffin Island, was also believed to be *Falco peregrinus*. A family of four birds was found frequenting a

high precipitous cliff at Fort Ross, Somerset Island, and the adult male was collected. *1939:* One seen chasing two ravens at Arctic Bay. On September 20, while the *Nascopie* was off the Labrador coast near Hamilton Inlet, an immature bird spent four or five hours on the ship, apparently tired out.

The Fort Ross and Arctic Bay records appear to be the most northerly for this species in Canada.

Lagopus lagopus (Linnaeus). WILLOW PTARMIGAN.—*1939:* Three were seen several miles inland from Churchill on August 7.

Marsh collected two at Eskimo Point on May 3, 1937.

Lagopus rupestris (Gmelin). ROCK PTARMIGAN.—*1938:* This species was not common. Three females, one with three young, the others with one each, were observed at Sugluk West, Que. An adult male was seen at Pond Inlet and a female with one young was noted at Arctic Bay. Adult and juvenile specimens were collected at Sugluk and Arctic Bay.

Bailey took two on May 12, 1930, at Lake Harbour and a female on an island off Markham Bay on July 3, 1930.

Learmonth collected a juvenile male at Fort Ross on August 25, 1940.

Fulica americana Gmelin. AMERICAN COOT.—Learmonth preserved a specimen which was found dead on sea ice at the east end of Bellot Strait on October 30, 1937. This must be considered an accidental occurrence as the bird was far north of the normal range for the species.

Charadrius hiaticula Linnaeus. GREATER RINGED PLOVER.—*1938:* Found only on a gravelly bar at the mouth of the Salmon River, Pond Inlet, Baffin Island. Three immatures, of which two were collected, were observed. *1939:* Individuals believed to be of this species were seen at River Clyde.

Charadrius semipalmatus Bonaparte. SEMIPALMATED PLOVER.—*1938:* Plentiful throughout the region of Hudson Straits and north to Pangnirtung. Five adult and five juvenile specimens were taken, representing the following localities: Lake Harbour, Wakeham Bay, Sugluk West, Port Harrison, and Pangnirtung. *1939:* A common species, noted at the following posts: about 15 at Cape Dorset, four at Southampton Island, one at Port Harson, common at Churchill, 10 at Chesterfield, and one at Cape Wolstenholme. An individual which may have been of this or the preceding species was seen at Fort Ross.

Learmonth's specimen of this species, taken on August 26, 1940, at Fort Ross, is of interest in view of the occurrence of the greater ringed plover in similar latitudes on adjacent Baffin Island. It also appears to represent a northward extension in the known range of the semipalmated plover. The specimen is a juvenile male.

Marsh secured a male at Eskimo Point on July 5, 1937.

Pluvialis dominica (Müller). GOLDEN PLOVER.—*1939:* Eight seen at close range on Southampton Island.

Squatarola squatarola (Linnaeus). BLACK-BELLIED PLOVER.—Learmonth took a pair at Cresswell Bay on June 16, 1938.

Arenaria interpres (Linnaeus). COMMON TURNSTONE.—*1938:* An adult and two downy young were collected at Coral Harbour, Southampton Island. Many small flocks were observed at Craig Harbour. Four juvenile specimens were collected. *1939:* Six, of which two were young that were unable to fly, were seen at Southampton Island. Several observed at Churchill.

A pair was collected at Cresswell Bay by Learmonth on June 17, 1938.

Phaeopus hudsonicus (Latham). HUDSONIAN CURLEW.—*1938:* Seen only at Churchill, Man. Several adult birds observed there on August 6 were highly agitated, probably concerned with their young concealed in the grass. One of the agitated birds was collected and proved to be a female. On the same date fairly large flocks of Hudsonian curlew were seen along the Churchill River. *1939:* About 30 were seen at Southampton Island, two at Churchill, and five at Chesterfield.

Totanus melanoleucus (Gmelin). GREATER YELLOWLEGS.—*1938:* About 50 birds of this species were observed at Churchill, in flocks of from 3 to 10. A female was collected on August 6.

Totanus flavipes (Gmelin). LESSER YELLOWLEGS.—*1938:* A small flock was observed on August 6 along the river near Churchill and several were seen in the townsite slough. *1939:* Several seen in small ponds near Churchill.

Calidris canutus Linnaeus. KNOT.—Learmonth collected a female on June 14 and a male on June 16, 1938, at Cresswell Bay.

Arquatella maritima (Brünnich). EASTERN PURPLE SANDPIPER.—*1938:* A small flock seen at Pond Inlet. There were many immatures at River Clyde. Three males and two females were collected at Clyde. *1939:* One seen at Fort Ross, Somerset Island.

Bailey collected two at Lake Harbour on June 1, 1932.

Learmonth secured a male on June 14 and a female on June 15, 1938, at Cresswell Bay.

Pisobia melanotos (Vieillot). PECTORAL SANDPIPER.—*1938:* An adult male was collected from a flock of 12 at Churchill.

Pisobia fuscicollis (Vieillot). WHITE-RUMPED SANDPIPER.—*1938:* The most commonly observed wader except the semipalmated sandpiper for the region as a whole during this year, even greatly exceeding the latter north of Hudson Straits. Three specimens were collected at Coral Harbour, Southampton Island, including a semidowny chick and a fully feathered juvenile. Juveniles were also taken at Chesterfield, N.W.T., River Clyde, and Arctic Bay. The latter was the most northerly observation. *1939:* Two were seen at Cape Dorset, five at Southampton Island, and two at Pond Inlet.

Learmonth secured two at Fort Ross on August 26, 1940.

Pisobia bairdi (Coues). BAIRD'S SANDPIPER.—*1938:* Several adults were seen and one collected at Chesterfield. Two juveniles, with down still adhering to the occiput were flushed from a small inland pool at Arctic Bay and one was collected.

Learmonth collected two specimens at Fort Ross on August 26 and September 11, 1940.

Pisobia minutilla (Vieillot). LEAST SANDPIPER.—*1938:* Seen only at Churchill about the townsite slough on August 6.

Pelidna alpina (Linnaeus). DUNLIN.—*1938:* This species was found in large numbers at Churchill and Chesterfield and specimens in the very handsome juvenile plumage were collected at both places. *1939:* Two seen at Chesterfield.

Limnodromus griseus (Gmelin). DOWITCHER.—*1938:* This species was migrating in fairly large numbers at Churchill and two juvenile specimens were taken. *1939:* One observed at Churchill near Fort Prince of Wales.

Micropalama himantopus (Bonaparte). STILT SANDPIPER.—*1938:* A flock of six juveniles was observed at Churchill. Two specimens collected were both females. *1939:* Several observed at Churchill.

Ereunetes pusillus (Linnaeus). SEMIPALMATED SANDPIPER.—*1938:* A very common shorebird on Southampton Island and on both coasts of Hudson Bay. A family of downy young was collected on Southampton Island and semidownies were taken at Port Harrison and Chesterfield. *1939:* This species and the semipalmated plover were the most widely distributed shorebirds observed during the trip of this year. Observed as follows: three at Southampton Island, one at Port Harrison, several at Churchill, common at Chesterfield, and about six at Fort Ross.

Crocethia alba (Pallas). SANDERLING.—*1938:* One seen at Pond Inlet. *1939:* Two seen on the sandy beach at Clyde River.

Learmonth secured a male on June 15 and a female on June 17, 1938, at Cresswell Bay. He also collected two at Fort Ross on August 26, 1940.

Phalaropus fulicarius (Linnaeus). RED PHALAROPE.—*1938:* Encountered in some numbers on Southampton Island, where two adult males and two partly feathered downy young were collected. Also several were seen at Chesterfield, where a juvenile was taken.

Bailey collected specimens at Lake Harbour on June 15, 1932, and a pair on Jubilee Island on June 29, 1930.

Learmonth secured specimens at Cresswell Bay about the middle of June, 1938.

Lobipes lobatus (Linnaeus). NORTHERN PHALAROPE.—*1938:* Five seen at Churchill. *1939:* Very common at Churchill. A flock of six seen at sea near Lake Harbour on August 14 were believed to be this species.

Stercorarius parasiticus (Linnaeus). PARASITIC JAEGER.—*1938:* A flock of eight seen at Southampton Island. Also noted in some numbers at Churchill, Chesterfield, and Craig Harbour and at Thule, Greenland. Two adults were collected. *1939:* Two were seen at Southampton Island and one at Churchill. Two observed at Chesterfield were chasing a snowy owl.

Learmonth collected two light-phased birds on June 17, 1938, at Cresswell Bay and a dark female on June 8, 1940, at Port Leopold.

Stercorarius longicaudus Vieillot. LONG-TAILED JAEGER.—*1938:* Two adults seen at Cape Dorset and two at Coral Harbour, Southampton Island. An adult was collected at Fort Ross, Somerset Island. *1939:* On July 19 at Cape Chidley and in Gray Strait there were at least one hundred and fifty. One was seen at Southampton Island; one at sea,

south of Southampton Island, on August 12; six seen near a large iceberg in Davis Straits off Frobisher Bay on August 17; one in Prince Regent Inlet on August 27; and one at Pond Inlet.

Learmonth collected males of this species, one on June 18, 1938, at Cresswell Bay and one in July, 1939, at Port Leopold.

Larus hyperboreus Gunnerus. GLAUCOUS GULL.—*1938:* The commonest gull in the area covered. It was observed at all ports of call except Churchill, Chesterfield, and River Clyde. A breeding colony of about 20 pairs was observed on the high, inaccessible cliffs near Glasgow Falls, Lake Harbour, on July 20. A male was collected at Craig Harbour. This bird possessed a vague, pinkish blush on the underparts, strongest on the breast. *1939:* The commonest and most widespread gull of the trip. Observed at nearly every port as follows: a few at Hebron, some at Cape Chidley; a few at Lake Harbour, Wakeham Bay, Sugluk West, Cape Dorset, Wolstenholme, Southampton Island, and Port Harrison; observed occasionally in Davis Straits and Baffin Bay; a few at Craig Harbour; adults and immatures at Pond Inlet and Pangnirtung; observed at Hebron, September 18.

Bailey collected a male on June 1, 1931, at Lake Harbour and a female off Big Island on June 18, 1930. He also collected eggs almost ready to hatch on an island off Big Island on June 18, 1930.

Learmonth collected a specimen at Port Leopold in July, 1939, and two at the same place on June 8, 1940.

Larus marinus Linnaeus. GREAT BLACK-BACKED GULL.—*1938:* The only northern observation of this species was that of an individual at Hebron, Labrador, in September. *1939:* Not seen north of Belle Isle Strait, where they were rather common.

Larus argentatus Brünnich. HERRING GULL.—*1938:* Fairly common. All those in Hudson Bay and Hudson Strait that could be scrutinized closely seemed to have the dark wing tips of *L. argentatus smithsonianus*. Specimens in fully adult plumage collected at Fort Ross and Arctic Bay were typical of the northern form, *L. argentatus thayeri* Brooks. These two birds possessed light *brown* irides and *purplish-pink* eyelids. *1939:* A few seen at Lake Harbour on July 22; a few at Southampton and many at Churchill and Chesterfield, these all being of the race *L. argentatus smithsonianus* Coues. *L. argentatus thayeri* was observed as follows: two were seen in ice pack in Prince Regent Inlet on August 29; many, of which 8 or 10 were young and unable to fly, at Fort Ross; about 15, of which a few were young birds, at Arctic Bay; and four at Pond Inlet.

Learmonth collected several specimens of the northern form, *L. argentatus thayeri*, including breeding adults and juveniles at Port Leopold and Fort Ross.

Larus kumlieni Brewster. KUMLIEN'S GULL.—*1938:* Gulls which were almost certainly of this species were seen near Cape Wolstenholme on July 29 and on August 14. Information to the effect that these birds nested near Erik Cove was secured locally. *1939:* A few were seen in the harbour at Wolstenholme on July 28 and August 13.

Larus philadelphia (Ord.). BONAPARTE'S GULL.—*1938:* A few seen at Churchill. *1939:* Common in the harbour at Churchill.

Pagophila alba Gunnerus. IVORY GULL.—*1938:* A few were seen in Lancaster Sound and Prince Regent Inlet and one was observed with glaucous gulls at Craig Harbour. *1939:* Two seen at Craig Harbour and two in Prince Regent Inlet on August 27.

Learmonth reports this species as quite abundant in spring but very scarce in summer on south Somerset Island. He collected two females on July 6, 1938, and two on June 22, 1940, at Port Leopold.

Rissa tridactyla (Linnaeus). KITTIWAKE.—*1938:* A fairly common species. Observed along the Labrador and Davis Strait as far north as Devon Island. *1939:* Observed commonly. A few in Belle Isle Strait, off the Labrador coast and around Cape Chidley; (not seen in Hudson Strait or Bay); a few in Davis Strait and Baffin Bay; several at Craig Harbour; a few in Lancaster Sound and Prince Regent Inlet; two at Pond Inlet; several at Clyde Inlet; a few in Cumberland Sound; common off the Labrador coast on the return trip, especially off Hamilton Inlet.

Learmonth collected this species at Bellot Strait on July 6, 1938, and at Port Leopold on June 23, 1939, and June 10, 1940.

Xema sabini (Sabine). SABINE'S GULL.—*1939:* Three seen at Southampton Island and one off Clyde Inlet on September 7.

Bailey secured a specimen during the last week of July, 1932, on Big Island.

Sterna paradisaea Brünnich. ARCTIC TERN.—*1938:* Common at Southampton Island and at Churchill; a few seen at Chesterfield; not noted elsewhere. *1939:* Abundant at Southampton Island; common in Churchill harbour; about eight were seen at Chesterfield.

Two taken at Cresswell Bay by Learmonth on June 18 and 23, 1938; also a specimen collected at Bellot Strait on June 23, 1939.

Alca torda Linnaeus. RAZOR-BILLED AUK.—*1938:* Last seen off the Labrador coast near Cartwright.

Uria aalge (Pontoppidan). COMMON MURRE.—*1938:* Not identified north of Belle Isle Strait. *1939:* Common in Belle Isle Strait and a few off the lower Labrador near Cartwright.

Uria lomvia (Linnaeus). THICK-BILLED MURRE.—*1938:* Common. Observed in Hudson Strait almost to the western entrance but not seen in Hudson Bay. Observed in Prince Regent Inlet, Admiralty Inlet, Lancaster Sound, and Baffin Bay north to Thule, Greenland. *1939:* Common at Cape Chidley and throughout Hudson Strait (but none in Hudson Bay) and northward in Davis Strait and Baffin Bay; a few seen at Craig Harbour; many in Prince Regent Inlet; a few in Lancaster Sound, near Pond Inlet; and at Clyde River. Murres unable to fly were seen in Prince Regent on August 27. Some of these were young, others may have been adults in the eclipse moult.

Bailey collected two specimens at Lake Harbour.

Three examples were taken by Learmonth in June and July, 1938 and 1939, on the southeast coast of Somerset Island, and five at Port Leopold on June 10, 1940.

Alle alle (Linnaeus). DOVEKIE.—*1938:* Abundant in Davis Strait and Baffin Bay during the latter part of August. Observed as far west as Lancaster Sound, about 87° west longitude. *1939:* First seen in Baffin Bay, east of Devon Island, on August 21; common in Prince Regent Inlet on August 31; some seen in Lancaster Sound and Admiralty Inlet. Flocks of 20 to 40 seen in Davis Strait near Clyde River but none south of Clyde.

Cepphus grylle (Linnaeus). BLACK GUILLEMOT.—*1938:* Distributed about as the last, but the most northerly observation was off Devon Island in Davis Strait. A female was collected at Pond Inlet. *1939:* Rather common and widespread, having been seen as follows: a few near Hebron; a few at Cape Chidley; one at Cape Smith; several at Port Burwell on August 16; one at Craig Harbour; a few in Lancaster Sound on August 26; several in Prince Regent Inlet on August 27. It was reported to winter at the edge of the ice-floe in Admiralty Inlet and Pond Inlet.

Bailey found this bird common about Lake Harbour and collected six breeding specimens. He secured an egg from a hollow in rough gravel far under a thin shelf of rock on July 3, 1930, on an island 25 miles west of Crooks Inlet.

Learmonth collected specimens at Port Leopold on June 15 and 27, 1939, July 6, 1938, and in May and June, 1940.

Fratercula arctica (Linnaeus). ATLANTIC PUFFIN.—*1938:* The most northern observation was off Rigolet, Labrador. *1939:* Some in Belle Isle Strait and one seen off Cartwright, Labrador, on July 12.

Nyctea nyctea (Linnaeus). SNOWY OWL.—*1938:* One adult was seen near Pond Inlet, Baffin Island. *1939:* One seen at Chesterfield and one immature specimen, on an ice-floe in Prince Regent Inlet on August 27.

Bailey took one at Lake Harbour on September 29, 1930, and also collected a young male from a nest at Lake Harbour in June, 1930.

Marsh collected specimens on February 28, May 20, and June 1, 1936, at Eskimo Point.

Asio flammeus (Pontoppidan). SHORT-EARED OWL.—*1939:* One seen several miles inland from Churchill.

Marsh collected a specimen on September 1, 1937, at Eskimo Point. He comments that the species is not common there.

Otocoris alpestris (Linnaeus). HORNED LARK.—*1938:* Observed north to Fort Ross and Arctic Bay, at which points it was as common as farther south. Seventeen specimens were taken as follows: two at Port Burwell, four at Lake Harbour, three at Sugluk West, four at Port Harrison, two at Arctic Bay, and two at Fort Ross. *1939:* One at Port Harrison; common at Churchill; one immature on ice-floe near ship in Prince Regent Inlet on August 28; about eight at Fort Ross and 10 at Arctic Bay.

Bailey took a specimen on May 22, 1930, at Lake Harbour.

Learmonth collected specimens at Cresswell Bay on June 16, 1938, and at Fort Ross on August 26, 1940.

Iridoprocne bicolor (Vieillot). TREE SWALLOW.—*1938:* A single bird was seen near the post at Chesterfield on August 10. It flew off, high over the water, in a southerly direction and could not be collected. *1939:* Four seen around small ponds in the townsite at Churchill on August 7.

Corvus corax Linnaeus. NORTHERN RAVEN.—*1938:* Not common, but a few seen at Hebron and Wakeham Bay and many at Pond Inlet. Individuals were seen at Lake Harbour, Craig Harbour, Port Burwell, and Cape Dorset. One was collected at Pond Inlet. *1939:* One at Lake Harbour, July 23; six at Cape Smith; one at Craig Harbour; two at Arctic Bay; three at Pond Inlet; about six at Pangnirtung; and one at Hebron, on September 18.

Sialia currucoides (Bechstein). MOUNTAIN BLUEBIRD.—*1938:* A male was collected from a small clump of spruces a few miles south of Churchill on August 6. This is the first definite record of the species at Churchill, but suggests the identity of a young bluebird taken by Twomey (9) on June 10, 1931, but lost before it could be identified.

Oenanthe oenanthe (Linnaeus). WHEATEAR.—*1938:* A family group was observed about half a mile from Cape Wolstenholme, Que., on August 14, and a specimen in the scale-marked juvenile plumage was taken. Immatures were observed at Arctic Bay and Pangnirtung, Baffin Island. Two were collected at Arctic Bay and one at the latter post. Constable Fitzrandolph, who was stationed at Lake Harbour, showed me on August 17 where a pair had nested in a rock-faced terrace near the Royal Canadian Mounted Police post earlier in the summer. The frequency with which this species was encountered in 1938 during the hurried *Nascopie* cruise would indicate that an increase in the number of wheatears has taken place in Baffin Island in the last 10 years (1). Soper writes (6) that this species was ". . . . . one of the rarest birds of Baffin Island."

Anthus spinoletta (Linnaeus). AMERICAN PIPIT.—*1938:* The most abundant land bird of the high rocky coastal region about Hudson Strait. Eleven specimens were taken, including juveniles at Cape Dorset, Lake Harbour, and Port Harrison. Other localities where specimens were taken were Port Burwell and Cape Wolstenholme. *1939:* Found rather commonly at most ports of call. Common at Hebron, Lake Harbour, Sugluk, Harrison, and Churchill. Several at Port Burwell, Wakeham Bay, Cape Dorset, Wolstenholme, Cape Smith, and Chesterfield. Four were definitely identified at Fort Ross; two at Arctic Bay, four at Pond Inlet, and about four at Pangnirtung.

Dendroica virens (Gmelin). BLACK-THROATED GREEN WARBLER.—*1938:* A male came aboard the *Nascopie* while the ship was out of sight of the coast of Labrador, about latitude 53° North, on September 17. The bird was in an exhausted condition, and though given food and water, died the next day.

Acanthis hornemanni (Holboell). ARCTIC REDPOLL.—*1938:* Observed only at Churchill, where an adult male and a juvenile were taken.

Acanthis linaria (Linnaeus). REDPOLL.—*1938:* Observed only at Sugluk West, Hebron, and Pangnirtung. A newly made nest was found at Hebron on July 15. Two adult females and two juveniles collected at Sugluk are typical *linaria*, while a female taken at Pangnirtung is *rostrata*. It is decidedly buffy in colour, indicating immaturity, and has a wing 80 mm. in length.

Passerculus sandwichensis (Gmelin). SAVANNAH SPARROW.—*1938:* Two adult specimens were collected at Wakeham Bay. The only other locality where this species was noted was Churchill, where it was common and two specimens were collected. *1939:* A few seen at Sugluk, two at Port Harrison, and several at Churchill.

Spizella arborea (Wilson). TREE SPARROW.—*1938:* Met with only at Churchill, where three juveniles and one adult were collected on August 6.

Zonotrichia leucophrys (Forster). WHITE-CROWNED SPARROW.—*1938:* This species was common at Hebron, Labrador, inhabiting the scrub willows. On July 15 many juveniles just able to fly were observed and two were caught by hand. One singing male was seen at Port Burwell, N.W.T., but could not be secured. *1939:* At Hebron a few adults were seen on July 17 and a nest with five eggs was found. A few seen at Lake Harbour on July 23; at Churchill it was common and one was heard singing at Hebron on September 18.

Calcarius lapponicus (Linnaeus). LAPLAND LONGSPUR.—*1938:* This was an abundant species at nearly all ports, but was not observed at Hebron, Lake Harbour, Port Burwell, or Wakeham Bay. An adult male was collected at Craig Harbour, Ellesmere Island. It was the only one seen there and appears to represent a northern record for the species. Twenty-two specimens were taken, including juveniles on the breeding grounds at Cape Dorset, Fort Harrison, and Southampton Island. *1939:* Found to be abundant at Southampton Island; a few seen at Churchill; common at Chesterfield; one seen on the ice in Prince Regent Inlet, about 30 miles from Fort Ross, on August 28; six at Arctic Bay; two at Pond Inlet; four at Clyde River; a few at Pangnirtung; and an immature came aboard the ship on September 16 in Davis Strait.

Bailey secured specimens on May 14 and 16, 1930, at Lake Harbour.

Marsh took a female at Eskimo Point on July 5, 1936.

Learmonth got this species on June 18, 1938, at Cresswell Bay and on June 21 at Bellot Strait. A common bird on Somerset Island.

Calcarius pictus (Swainson). SMITH'S LONGSPUR.—*1938:* An adult female and a juvenile collected at Churchill on August 6. *1939:* Several birds observed in company with Lapland longspurs about the gravel flats in the townsite of Churchill.

Plectrophenax nivalis (Linnaeus). SNOW BUNTING.—*1938:* The most abundant species of the trip. It was noted at every port with the exception of Hebron. Seventeen specimens, representing a variety of plumages, were taken. *1939:* The most commonly observed land bird, being seen at every port except Hebron and Wakeham Bay.

Bailey collected specimens at Lake Harbour on May 22, 1930. He also collected two sets of eggs at Lake Harbour on July 2 and 23, 1930.

Learmonth found the snow bunting common on Somerset Island and took examples on June 20, 1938, at Cresswell Bay and on September 10 and 11, 1940, at Fort Ross.

References

1. FORBES, J. R. Recent observations on the Greenland wheatear. Auk, 55 : 492-495. 1938.
2. KENNARD, F. H. The specific status of the greater snow goose. Proc. New England Zool. Club, 9 : 89. 1927.
3. PREBLE, E. A. A biological investigation of the Hudson Bay region. North Am. Fauna, No. 22 : 76. 1902.
4. SNYDER, L. L. Pied-billed grebe from Baffin Island. Can. Field Nat. 49 : 123. 1935.
5. SNYDER, L. L. On the Hudson Bay eider. Occasional Papers, Roy. Ontario Museum Zool., No. 6. 1941.
6. SOPER, J. D. A faunal investigation of southern Baffin Island. Bull. Natl. Museum Can., No. 53 : 116. 1928.
7. TAVERNER, P. A. A study of *Branta canadensis* (Linnaeus), the Canada goose. Ann. Rept. Natl. Museum Can., 1929 : 34. 1931.
8. TAVERNER, P. A. Fieldfare, an addition to the American list, and some Arctic notes. Auk, 57 : 119. 1940.
9. TAVERNER, P. A. and SUTTON, G. M. The birds of Churchill, Manitoba. Ann. Carnegie Museum, 23 : 67. 1934.

EFFECTS OF PHENOTHIAZINE ON HORSES

I. STUDIES IN HAEMATOLOGY AND PHARMACOLOGY[1]

By W. E. Swales[2], H. B. Collier[3], and Della Allen[4]

Abstract

Studies have been made on the anaemia and cythaemolytic icterus produced by 60 and 70 gm. doses of phenothiazine to horses weighing approximately 1200 lb. The continued and prolonged haemolytic effect of a single dose may be serious in a horse having a large proportion of microcytic erythrocytes. No evidence of damage to the liver was obtained, but clinical observations confirm a previous report that severe injury to the urinary system may result from a dose of 60 gm. of phenothiazine to a 1200 lb. horse.

A dose of 30 gm. removed all the strongyloid nematodes and did not cause haemolysis. Less than half the dose could be recovered from the excreta of treated animals. The effects of phenothiazine on horses are similar to those of sulphanilamide and sulphapyridine in susceptible humans. The possibility of over-stimulation of the spleen with the consequent hyperactivity causing prolonged haemolysis is mentioned.

The development of phenothiazine as an anthelmintic has led to trials with all domestic animal species. The high efficiency of this chemical against the strongyloid nematodes in the caecum and large intestine of horses was determined by Harwood, Habermann, Roberts, and Hunt (8) and later by Grahame, Morgan, and Sloane (6), Knowles and Franklin (10), and Errington and Westerfield (3). Although all these studies confirmed the efficiency of phenothiazine, there was no general agreement regarding its toxicity for horses. The descriptions of toxic symptoms were at first confined to the mention of bronzed mucous membranes, slight malaise, abdominal pain, and anaemia. More detailed studies were later described by Errington (2).

The work described in this paper has been confined to observations on two horses, but a great many data on haematological and chemical findings have been obtained and are deemed worthy of publication at this time.

The haemolytic anaemia that follows administration of relatively large doses of phenothiazine to horses is of considerable interest, particularly because of its relationship to the anaemias sometimes brought about by sulphonamide therapy (5). An anaemia following the oral administration of phenothiazine to man, rats, and rabbits was first recorded by Thomas *et al.* (15). It is probable that the equine anaemia described herein was similar to that in other mammals.

[1] *Manuscript received June 29, 1942.*

Contribution from the Division of Animal Pathology, Science Service, Dominion Department of Agriculture, and the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Associate Animal Pathologist, Division of Animal Pathology, Science Service, Dominion Department of Agriculture; Institute of Parasitology, Macdonald College, Que.*

[3] *At the time, Lecturer, Institute of Parasitology, McGill University, Macdonald College, Que. At present, Assistant Professor of Biochemistry, Dalhousie University, Halifax, N.S.*

[4] *Research Assistant, Institute of Parasitology, McGill University, Macdonald College, Que.*

Experiment I

Subject

Horse *A*, six years old, gelding; good health and condition; weight 1250 lb.

Procedure

Normal levels of haemoglobin were obtained on five occasions over a period of 28 days by the Dare haemoglobinometer. Erythrocyte, leucocyte, and strongyloid nematode counts were made by standard methods. Seventy grams of pure phenothiazine were administered in the feed, after which all faeces and urine were collected separately for 80 hr. Blood samples, commencing at two hours after dosing, were collected at intervals for 34 days; from these samples the bilirubinaemia was measured by the indirect Van den Bergh method as modified by Malloy and Evelyn (11) to give more complete recovery of pigment; the haematological measurements were made as before.

The results are presented in Table I. This preliminary experiment served to show, and measure the intensity of, the anaemia and apparent cythaemolytic icterus produced in this animal. The anthelmintic effect was completely efficient; worms were evacuated in the faeces between the 24th and 48th hours as follows:

Hours after treatment	Worms passed (estimated)	
	Cylicostomes	Large Strongyles
24 – 26	37,200	31
26 – 29	9900	42
29 – 48	Very few	10

The quantitative tests for nematode eggs dropped from the average pre-treatment figure of 1460 eggs per gram to zero on the second day after treatment. All tests remained negative for 18 days and no quantitative test was positive until two months after treatment.

The clinical effects of the treatment were not severe. At the end of 24 hr., frequent and apparently painful urination occurred and there was a noticeable decrease in appetite. The normal 24 hr. faecal output of 48 lb. was reduced to 35 lb., but returned almost to normal on the second day. The mucous membranes of the eye and mouth were definitely bronzed 48 hr. after treatment and remained coloured for 16 days. The horse was not distressed by a run of about two miles on the fourth day following treatment.

The Recovery of Phenothiazine and Derivatives

Phenothiazine and its derivatives in the faeces, blood, and urine were estimated by the methods previously described (13), with certain modifications. Hydrogen peroxide was found to be unsuitable for oxidation of leucophenothiazone to the red pigment, and ferric chloride was substituted for it. One drop of 1% ferric chloride in 7*N* hydrochloric acid was added to the acid solution in the colorimeter tube and a reading taken. Then the pigment

TABLE I

HORSE *A*, EXPT. I

Changes in blood levels following a dose of 70 gm. of phenothiazine

	Haemoglobin		Erythrocytes		Bilirubin, mg., %
	Gm., %	Change, %	Millions/ mm.^{3}	Change, %	
Pretreatment normals	11.2 (σ = 0.1)		6.9 (σ = 0.5)		
Post-treatment values after:					
2 hr.	11.2	0	6.6	− 4	1.4
1 day	11.2	0	6.7	− 3	3.1
2 days	10.5	− 6	6.6	− 4	4.7
3 "	10.4	− 7	6.2	−10	3.7
4 "	10.5	− 6	5.5	−20	3.5
6 "	9.6	−14	5.8	−16	3.5
7 "	9.6	−14	5.7	−17	3.3
8 "	9.9	−12	5.7	−17	3.3
10 "	9.9	−12	5.7	−17	2.9
11 "	9.3	−17	5.8	−16	3.3
12 "	9.4	−16	5.4	−22	3.0
13 "	9.0	−20	5.0	−28	3.3
15 "	8.8	−21	4.5	−35	3.3
20 "	—	—	5.1	−26	1.9
24 "	9.8	−13	5.5	−20	1.6
27 "	10.3	− 8	6.3	− 9	1.3
34 "	11.0	− 2	6.9	0	0.9

was bleached by addition of a small crystal of stannous chloride and a second reading taken to represent the blank. The difference was calculated as phenothiazone.

This method appeared satisfactory for blood, except when highly jaundiced. In such cases, changes took place in pigments other than phenothiazone. When normal sheep urine was treated as above mentioned, no colour change was observed, but normal horse urine contained a substance that gave a purplish colour with ferric chloride and was reduced by stannous chloride. Unfortunately, since the concentration of this chromogen in normal horse urines was not constant, it was impossible to establish satisfactory blank values. An average blank has been deducted from the tabulated figures for urine, but the total must be regarded as only approximate, although the error is believed to be less than 10% of the total output of phenothiazone.

In the faeces, traces of oxidation products were found to be present. An aliquot sample of dried and ground faeces was extracted with boiling water under quantitative conditions, and a suitable volume of this extract was acidified and treated with ferric chloride and stannous chloride, as described above, in order to estimate the phenothiazone content. Normal faeces gave no significant reaction, under these conditions.

These analyses resulted in the recovery of the amounts shown in Table II. The discrepancy between the amounts recovered from the horse and the sheep (13) could not be explained; the possibility of a large percentage being retained in host tissues was considered.

TABLE II

HORSE *A*, EXPT. I

Detection and recovery of phenothiazine and oxidation derivatives

Blood serum

Post-treatment times, hr.	Total leucophenothiazone, %
5½	0.0060
24	0.0035
49	0.0010
77	0.0006

Faeces

Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		Oxidation products (as leucophenothiazone), gm.
		Gm.	Gm./hr.	
7 – 22	2593	7.57	0.50	2.80
22 – 23	334	1.12	2.24	0.45
23 – 28	512	1.24	0.24	0.52
28 – 31	490	1.23	0.61	0.36
31 – 47	2860	1.56	0.10	1.37
47 – 49	418	0.28	0.14	0.14
49 – 55	1038	0.35	0.06	0.52
		Total = 13.4		Total = 6.16 = 5.7 gm. phenothiazine

Urine

Post-treatment periods, hr.	Volume, ml.	Total leucophenothiazone		
		%	Gm.	Gm./hr.
0 – 1	1060	0.004	0.05	0.05
1 – 9	975	0.181	1.77	0.22
9 – 24	2000	0.268	5.35	0.36
24 – 33	1030	0.151	1.56	0.22
33 – 49	1100	0.099	1.09	0.07
49 – 58	1000	0.035	0.35	0.04
58 – 72	1100	0.043	0.48	0.03
72 – 80	1050	0.016	0.17	0.02
Washings	1230	0.058	0.71	—
			Total = 10.8 = 10.0 gm. phenothiazine	

NOTE: *Total recovery = 29.1 gm. phenothiazine (= 41.6% of dose).*

Experiment II

Subject

Horse *B*, five years old, gelding; good health and condition, weight approximately 1200 lb.

Procedure

Normal levels of haemoglobin, blood cells, blood bilirubin, erythrocyte volume, erythrocyte fragility, blood sedimentation rates, faecal output, and strongyloid nematode egg output were measured on several occasions over a period of two months. The accuracy of haemoglobin determinations was increased by the use of a calibrated acid haematin standard in a Klett-Summerson photoelectric colorimeter. The tests for erythrocyte fragility were made by using a series of sodium chloride solutions, 9.5 cc. of each, ranging from 0.85 to 0.50% in steps of 0.02; 0.5 cc. of blood was added to each tube and that percentage of sodium chloride that brought about a trace of haemolysis (2% as determined colorimetrically against a 100% distilled water check) was taken as the end point.

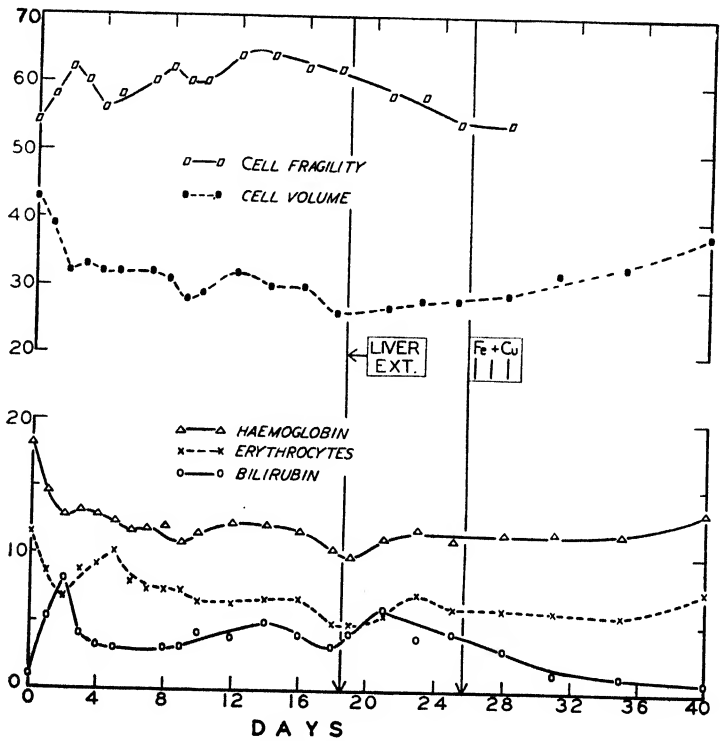


FIG. 1. *Blood values of Horse B for the period of 40 days following a dose of 60 gm. of phenothiazine. Cell fragility = % NaCl × 100; cell volume = %; haemoglobin = gm., %; erythrocytes = millions per cu. mm.; bilirubin = mg., %.*

The horse was dosed with 60 gm. of recrystallized phenothiazine in feed, which was readily consumed when a half pint of molasses was admixed. All the faeces were collected for five days and the urine for six days. Blood samples were taken at intervals for 40 days; the measurements made are shown in Table III and Figs. 1 and 2.

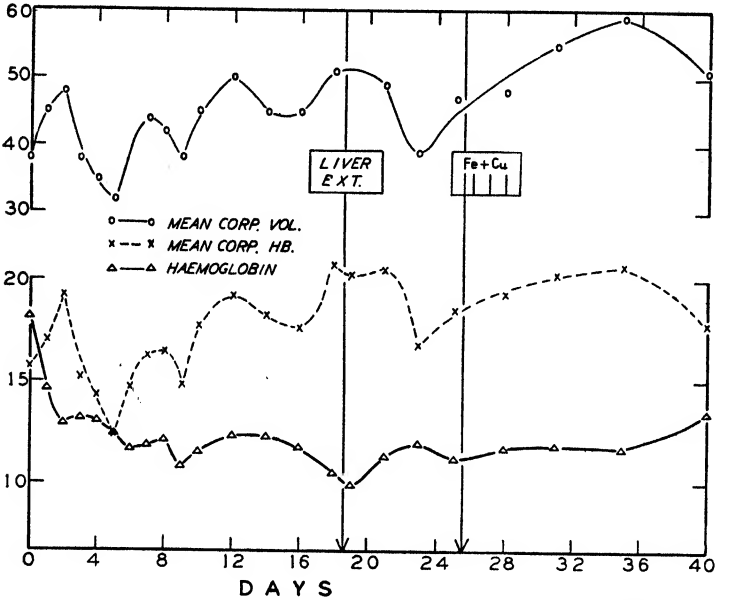


FIG. 2. *Further blood values of Horse B (same period as in Fig. 1). Mean corpuscular volume = μ^3; mean corpuscular haemoglobin = $\mu\mu$g.; haemoglobin = gm., %.*

In addition to the measurements shown in Table III observations were made on "vital stained" and Giemsa stained smears from each blood sample for the presence of nucleated erythrocytes and reticulocytes. The examinations for reticulocytes were made by the conventional "vital staining" method, using 1% cresyl blue in saline and also by the alcoholic cresyl blue staining of a wet smear (16). No true reticulocytes or nucleated erythrocytes were found during the experiment; however, on the seventh day after treatment a number of the erythrocytes had a small dark body in or just beneath the membrane. These bodies reached their maximum on the 12th day after treatment, when they were present in approximately 70% of the erythrocytes; after this they decreased until the 36th day, when none could be found. They were apparently nuclear fragments, and were comparable with the Howell-Jolly bodies of human erythrocytes*.

The faecal urobilinogen was determined by the method of Watson (16) in order to throw light upon the nature of the icterus. Table IV shows the

* *The recent finding of A. P. Richardson (Federation Proc. 1 : 164. 1942.), that Heinz bodies are formed during sulphanilamide anaemia, suggests that the bodies observed in the horse may be of a similar nature.*

TABLE III

HORSE *B*, EXPT. II

Blood changes following a dose of 60 gm. of phenothiazine

—	Erythrocytes		Haemoglobin		Mean corpuscular haemoglobin		Erythrocyte volume		Mean corpuscular volume		Cell fragility		Sedimentation rate				Bilirubin		Leucocytes	
													1st hour		2nd hour					
	Millions per mm.³	Change, %	Gm., %	Change, %	μμg.	Change, %	%	Change, %	μ³	Change, %	NaCl, %	Change, %	%	Change, %	%	Change, %	Mg, %	Change, %	Thousands per mm.³	Change, %
Pretreatment	11.5 (σ=0.3)		18.1 (σ=0.5)		15.8 (σ=0.5)		43 (σ=2)		38 (σ=2)		0.54 (σ=0.00)		37 (σ=9)		46 (σ=7)		1.1 (σ=0.2)		9.1	
Post-treatment																				
5 hr.	11.8	3	18.0	− 1	15.3	− 3	44	2	37	− 3	0.56	4	16	−57	23	−50	1.2	9	9.2	1
1 day	8.6	−25	14.6	−19	17.0	8	39	− 9	45	18	0.58	7	12	−68	27	−41	5.2	373	10.9	20
2 days	6.7	−43	12.9	−29	19.2	22	32	−26	48	26	0.62	15	—	—	—	—	8.0	628	16.8	85
3 days	8.7	−24	13.2	−27	15.2	− 4	33	−23	38	0	0.60	11	48	30	58	26	4.2	282	14.4	58
4 days	9.1	−21	13.0	−28	14.3	−10	32	−26	35	− 8	0.56	4	55	49	60	31	3.3	200	12.4	36
5 days	10.1	−12	12.4	−31	12.3	−22	32	−26	32	−16	0.58	7	57	54	61	33	3.1	182	8.3	− 9
6 days	7.9	−31	11.6	−36	14.7	− 7	—	—	—	—	—	—	—	—	—	—	—	—	8.6	− 6
7 days	7.3	−37	11.8	−35	16.2	3	32	−26	44	16	0.60	11	55	49	60	31	3.1	182	9.5	4
8 days	7.3	−37	12.0	−34	16.4	4	31	−28	42	11	0.62	15	53	43	59	28	3.1	182	10.7	18
9 days	7.3	−37	10.8	−40	14.8	− 6	28	−35	38	0	0.60	11	60	62	64	39	3.2	191	11.8	30
10 days	6.5	−43	11.5	−37	17.7	12	29	−33	45	18	0.60	11	55	49	61	33	4.4	300	9.3	2
12 days	6.4	−44	12.3	−32	19.2	22	32	−26	50	32	0.64	19	—	—	—	—	4.0	264	10.9	20
14 days	6.7	−42	12.2	−33	18.2	15	30	−30	45	18	0.64	19	55	49	60	31	5.0	354	13.5	48
16 days	6.6	−43	11.6	−36	17.6	11	30	−30	45	18	0.62	15	60	62	63	37	4.2	282	14.3	57
18 days	5.0	−57	10.4	−43	20.8	32	26	−40	51	34	0.62	15	65	76	68	48	3.3	200	14.0	54
Liver extract administered																				
19 days	4.9	−57	9.9	−45	20.2	28	—	—	—	—	—	—	—	—	—	—	4.3	291	—	—
21 days	5.5	−52	11.2	−38	20.4	29	27	−37	49	29	0.58	7	66	78	68	48	6.0	445	11.3	24
23 days	7.0	−39	11.9	−34	16.8	6	28	−35	39	3	0.58	7	66	78	67	46	4.0	264	8.2	−10
Iron therapy started (25)																				
25 days	6.0	−48	11.1	−39	18.5	17	28	−35	47	24	0.54	0	65	76	67	46	4.4	300	10.5	15
28 days	6.0	−48	11.6	−36	19.3	22	29	−33	48	26	0.54	0	65	76	67	46	3.1	182	7.7	−15
31 days	5.8	−50	11.7	−35	20.2	28	32	−26	55	45	—	—	—	—	—	—	1.4	27	7.9	−13
35 days	5.6	−51	11.6	−36	20.7	31	33	−23	59	55	—	—	—	—	—	—	1.2	9	—	—
40 days	7.5	−35	13.4	−26	17.8	13	38	−12	51	34	—	—	—	—	—	—	0.8	−27	—	—

data obtained. The normal faeces were free of urobilinogen and the relatively large amounts present following the treatment indicated that the icterus was not of an obstructive origin. The figures presented are arbitrary colorimetric units and do not represent absolute amounts.

TABLE IV

HORSE *B*, EXPT. II

Detection of urobilinogen in the faeces

Post-treatment periods	Colorimeter reading
Normal	0
18–24 hr.	90
3rd day	242
4th "	157
7th "	94
8th "	62
9th "	155
10th "	52
11th "	172
12th "	161
14th "	125
15th "	167
16th "	116
17th "	90
25th "	50
39th "	10

Direct Van den Bergh tests for uncombined blood bile pigments were made on the first three days after treatment. No immediate reactions occurred, thus there was no suggestion of hepatic dysfunction or even of a temporary inability of the polygonal cells to combine the pigment.

The continuation of the high indirect readings was noted as being important in consideration of the haemolysis.

Tests of the urine for bile pigments could not be made by colorimetric methods owing to interference from the thiazone dyes. Surface tension tests did not reveal significant amounts of bile.

Clinical effects on the urinary tract were definite and characterized by thirst, proportional increase in urine, frequent urination, and, between the second and fourth days, severe pain over the loins. There was a definite haematuria and a high protein content in the urine on the second day; the erythrocytes and casts were numerous and the trichloracetic acid test revealed masses of protein precipitate. The protein decreased suddenly on the fourth day, but numerous erythrocytes were present until the fifth day, when the clinical symptoms of urinary tract damage were no longer present.

On the 18th day after treatment the secondary anaemia had become quite severe and a trial intramuscular injection of 50 U.S.P. units of liver extract

TABLE V

HORSE *B*, EXPT. II

Excretion of phenothiazine and derivatives in faeces and urine

Faeces

Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		Oxidation products (as leucophenothiazone), gm.
		Gm.	Gm./hr.	
0 – 12	618	0.95	0.08	0.28
12 – 14½	660	0.56	0.22	0.20
14½ – 16½	285	0.68	0.34	0.24
16½ – 22	956	2.40	0.43	0.61
22 – 23½	406	1.75	1.17	0.34
23½ – 25½	620	1.20	0.27	0.65
25½ – 28				
28 – 30	333	0.84	0.42	0.29
30 – 36	467	1.25	0.20	0.33
36 – 47	410	0.72	0.07	0.23
47 – 50	423	0.46	0.15	0.24
50 – 52	455	0.24	0.12	0.14
52 – 54½	316	0.10	0.04	0.06
		Total = 11.2 gm.		Total = 3.6 = 3.3 gm. phenothiazine

Urine

Post-treatment periods, hr.	Volume, ml.	Total leucophenothiazone		
		Per cent	Gm.	Mg./hr.
0 – 4	1240	0.038	0.48	0.12
4 – 7½	930	0.204	1.89	0.54
7½ – 23	2270	0.256	5.82	0.38
23 – 31½	1880	0.202	3.80	0.45
31½ – 36	2230	0.022	0.49	0.09
36 – 48	3180	0.025	0.79	0.07
48 – 72	4455	0.020	0.89	0.04
72 – 76½	740	0.026	0.19	0.04
76½ – 80	630	0.022	0.14	0.04
80 – 86	1050	0.009	0.10	0.02
86 – 95	2070	0.008	0.18	0.02
95 – 101½	1310	0.010	0.13	0.02
101½ – 103½	1000	0.005	0.05	0.03
103½ – 119	2400	0.002	0.05	0.01
119 – 127½	2080	0.002	0.05	0.01
127½ – 143	2330	0.0003	0.01	—
Washings	11,340	0.0008	0.09	—
			Total = 15.2 = 14.0 gm. phenothiazine	

NOTE: *Total recovery = 28.5 gm. phenothiazine (= 47.5% of dose).*

was administered. The effects on the blood were not marked, although some improvement in the erythrocyte and haemoglobin levels could be noted (Fig. 1). On the 25th day a course of four daily doses of 4 gm. of ferrous sulphate with 0.1 gm. of copper sulphate was instituted. This treatment had no noticeable effect upon the trends.

In this experiment the anthelmintic effect was complete; nearly all the worm parasites (20,350 Cylicostomes, 577 *Strongylus* spp., and 175 *Habronema* spp.) were passed in the faeces between the 24th and 48th hours after treatment. A further recovery of 22 *Strongylus* spp. was made during the third and fourth days.

The previous tests for ova had remained fairly constantly at 2000 eggs per gram of faeces for four weeks; these tests dropped to zero on the second post-treatment day and did not become positive again for eight weeks. Qualitative tests (sugar flotation) remained negative for 14 post-treatment days, after which the findings varied from one to three eggs per slide for the next 30 days.

Table V shows the amounts of phenothiazine and oxidation derivatives from the excreta during the six days following treatment. Again it will be noted that less than 50% recovery was attained.

Experiment III

Horse *A* was again used for a test on the effects of a smaller dose, 97 days after it had been dosed previously. After the normal blood levels over a period of 16 days were determined, a dose of 30 gm. of pure phenothiazine was administered in the feed. No clinical effects could be noted and the blood examinations revealed only an insignificant haemolysis. The haematological and chemical data are presented in Tables VI and VII.

The anthelmintic effect was complete as were the effects from larger doses. A pretreatment quantitative count of 1200 eggs per gram immediately fell to,

TABLE VI

HORSE *A*, EXPT. III

The blood values following a dose of 30 gm. of phenothiazine

—	Erythrocytes, millions/mm.^{3}	Haemoglobin, gm., %	Erythrocyte volume, %	Cell fragility, % NaCl	Bilirubin, mg., %
Pretreatment normals	7.5(σ=0.5)	13.1(σ=0.5)	37.3(σ=1.5)	0.59(σ=0.00)	0.3(σ=0.1)
Values after treatment. Days					
1	8.5	15.3	38	0.57	0.6
2	8.6	15.2	39	0.58	0.5
3	6.9	14.0	37	0.60	0.3
4	8.4	14.7	40	0.58	0.3
5	7.7	13.2	—	—	0.2
7	7.7	14.2	37	0.60	0.3
14	7.5	13.9	38	—	0.3

TABLE VII

HORSE *A*, EXPT. III

Excretion of phenothiazine and derivatives in faeces and urine

Faeces

Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		Oxidation products (as leucophenothiazone), gm.
		Gm.	Gm./hr.	
0 – 16	360	0.03	0.002	0.02
16 – 16½	520	0.09	0.180	0.17
16½ – 20	460	0.24	0.068	0.20
20 – 22	324	0.28	0.140	0.15
22 – 24	445	0.36	0.180	0.24
24 – 28½	1580	0.39	0.086	0.65
28½ – 37	1120	0.06	0.007	0.21
37 – 40½	2480	0.12	0.048	0.20
40½ – 49	1330	0.00	0.000	0.13
		Total = 1.57		Total = 1.97 = 1.83 gm. phenothiazine

Urine

Post-treatment periods, hr.	Volume, ml.	Total leucophenothiazone		
		Per cent	Gm.	Gm./hr.
0 – 7	750	0.056	0.42	0.06
7 – 18½	2450	0.065	1.59	0.14
18½ – 26	1100	0.132	1.45	0.19
26 – 42½	3340	0.055	1.84	0.11
42½ – 66½	4000	0.014	0.64	0.03
			Total = 5.94 = 5.50 gm. phenothiazine	

NOTE: *Total recovery = 8.90 gm. phenothiazine = 29.7% of dose.*

and remained for 40 days at zero; 5061 strongyloid nematodes were recovered from the faeces during the first two post-treatment days.

Discussion

Experiment I confirmed the fact that a cythaemolytic icterus may be produced by a dose of 0.05 gm. of phenothiazine per pound of live weight in a horse and that the effect upon the blood may be prolonged. Failure to recover more than 41% of the dose through the intestinal and urinary tracts seemed to indicate either fixation of a large proportion of the dose in the body or excretion in some form which the methods could not detect. The deriva-

tive recovered from the urine was a conjugate, very similar to, if not identical with, the conjugate recovered from the urine of treated sheep (1).

Experiment II, with more detailed observations on blood changes, provides data on the nature of the anaemia and icterus. It is noted that the primary anaemia is brought about by haemolysis of the circulating erythrocytes, with a relatively small increase in fragility. It was noted during pretreatment observations that this horse had an unusually large number of erythrocytes (nearly 12,000,000/mm.^{3}) and that a large proportion was microcytes, the mean corpuscular volume being 37 as compared with 51 for horse *A*. According to C. P. Neser* regular exercise results in a marked increase in erythrocytes; horse *B* was in excellent physical condition and did not tire easily, even with violent exercise. Neser found that clinically healthy horses varied in erythrocyte numbers from 5.25 to 11.5 millions per mm.^{3}; he recorded a count of 12.5 millions per mm.^{3} in a highly trained racehorse. The upward trends of the mean corpuscular volume and mean haemoglobin content seem to indicate that the smaller erythrocytes (or microcytes) are more susceptible to the haemolysing agent; the partial recovery of the blood elements on the fifth day was accompanied by a reduction in the mean volume and mean haemoglobin. Again, in succeeding waves of haemolysis, the mean corpuscular volume and haemoglobin increased. Although tests of numbers of horses would, of course, be necessary for significance, this observation suggests that animals having a large proportion of microcytes (or low mean corpuscular volume) would be more susceptible to the haemolytic anaemia produced by phenothiazine therapy.

The recurring haemolysis, correlated with the lack of recovery of even half of the dose by the methods used, seemed to give an indication that the proportion of the drug not immediately excreted by the body at least in a recoverable form, continued to act as a haemolysing substance in the body tissues. Another possibility is that the spleen became overstimulated and destroyed erythrocytes by hyperactivity; the descriptions of splenomegaly by Errington (2), Hatcher (9), and others, lends some support to such a hypothesis. It is evident, however, that active erythropoiesis continued.

The relatively high levels of the bilirubinaemia, particularly following a fall in erythrocytes, the presence of faecal urobilinogen and the absence of a definite direct Van den Bergh reaction suggested haemolysis of circulating cells rather than dysfunction of the liver or haemopoietic organs. It is evident that there were severe pathological changes in the urinary system; this observation suggests that in horse *B*, changes similar to those described in detail by Errington (2) were present. Errington recorded haemoglobinuria but not haematuria, although he found erythrocytes in the tubules of damaged kidneys.

A series of experiments designed to show details of the mechanism of the haemolysis is in progress.

* *Neser, C. P. 9th and 10th Rept. Dir. Vet. Ed. Research, S. Africa, 481-558. 1924.*

References

1. COLLIER, H. B. Can. J. Research, D, 18(7) : 272-278. 1940.
2. ERRINGTON, B. J. Vet. Med. 36 : 188-193. 1941.
3. ERRINGTON, B. J. and WESTERFIELD, C. Vet. Med. 35 : 688-693. 1940.
4. FOLSE, C. D. Vet. Med. 36 : 430-431. 1941.
5. FOX, C. L., JR. and OTTENBERG, R. J. Clin. Investigation, 20 : 593-602. 1941.
6. GRAHAME, T., MORGAN, D. O., and SLOANE, J. E. N. Vet. Record, 52 : 660-663. 1940.
7. HABERMANN, R. T., HARWOOD, P. D., and HUNT, W. H. North Am. Vet. 22 : 85-92. 1941.
8. HARWOOD, P. D., HABERMANN, R. T., ROBERTS, E. H., and HUNT, W. H. Proc. Helminthol. Soc. Wash. 7 : 18-20. 1940.
9. HATCHER, W. L. North Am. Vet. 22 : 159-160. 1941.
10. KNOWLES, R. H. and FRANKLIN, A. V. Vet. Record, 52 : 663-664. 1940.
11. MALLOY, H. T. and EVELYN, K. A. J. Biol. Chem. 119(2) : 481-490. 1937.
12. MERIWEATHER, B. Vet. Med. 36 : 374-375. 1941.
13. SWALES, W. E. and COLLIER, H. B. Can. J. Research, D, 18(7) : 279-287. 1940.
14. TAYLOR, E. L., SANDERSON, K. M., *et al.* Vet. Record, 52 : 635-665. 1940.
15. THOMAS, J. O., MCNAUGHT, J. B., and DEEDS, F. J. Ind. Hyg. Toxicol. 20 : 419-427. 1938.
16. WATSON, C. J. Am. J. Clin. Path. 6 : 458-475. 1936.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 20, SEC. D. DECEMBER, 1942 NUMBER 12

MAIN ARTERIES IN THE REGION OF THE NECK AND THORAX OF THE AUSTRALIAN CASSOWARY[1]

BY FRED H. GLENNY[2]

Abstract

A mature specimen of the Australian cassowary (*Casuarius australis* Wallace) was dissected and a diagram of the main arteries in the neck region prepared. In the arrangement and distribution of these arteries, the cassowary differs from other species of birds previously studied. The left radix aortae and left ductus Botalli remain as a ligamentous vestige, the ligamentum aortae. The left internal carotid artery alone enters the hypapophysial canal. The right internal carotid artery is lacking or has become modified to form the ascending oesophageal artery of the adult, in the basal portion of the neck. The pattern of the arrangement of the main arteries in the neck and thorax shows a relatively primitive avian condition.

In several recent papers, the writer (4 to 8) has been able to demonstrate a large variety of arrangement patterns for the main arteries in the neck and thorax of birds of various orders and families. At the same time it has been possible to add in small part to the present knowledge of the gross anatomy of this class of vertebrates. The present paper deals with the distribution of the main neck and thoracic arteries and their branches in the cassowary (*Casuarius australis*).

In some minor respects, the arrangement of the main arteries in the neck and thorax resembles that found in the kiwi (*Apteryx australis mantelli*) but owing to the much greater size of the cassowary, a greater number of vessels are present to supply the various areas. The origin and distribution of some of the more important arteries of the neck differ in the two groups to such an extent that they may be regarded as having fundamental ordinal characters.

Huxley (9), in 1867, placed the cassowaries and kiwis in the same order, the Ratitae, but at the same time he recognized certain fundamental structural differences between the two groups. Later, however, Wetmore (11) divided the paleognathous birds into several groups and accorded ordinal rank to each.

In 1873, Garrod (3) reported on the carotid arteries of birds and remarked that in the Struthiones, both carotid arteries (left and right) were present in *Struthio camelus* Linné, *Casuarius bennetti* Gould, *Casuarius bicarunculatus* Sclater, and *Dromaeus novae-hollandiae* (Latham), whereas the left alone was

[1] *Manuscript received July 23, 1942.*

Contribution from the Department of Zoology, University of Toronto, Toronto, Ont.

[2] *Formerly Assistant, Department of Zoology, University of Toronto, Toronto, Ont. Now with the Royal Canadian Army Medical Corps.*

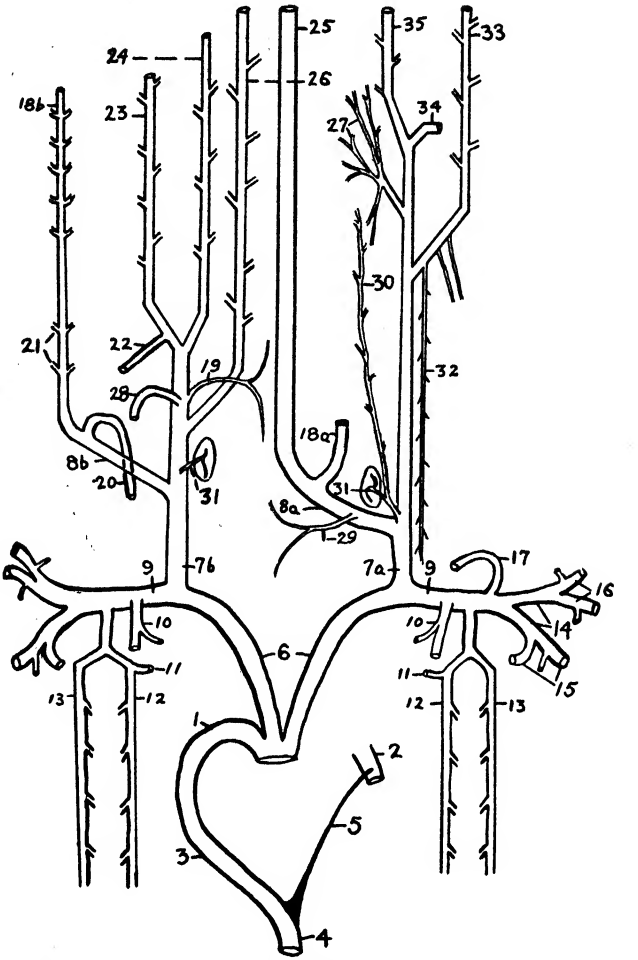


FIG. 1. *Diagrammatic representation of the main arteries in the neck and thorax of the Australian cassowary (Casuarius australis). Ventral view.*

KEY TO ABBREVIATIONS

(*1*) Right systemic (fourth aortic) arch; (*2*) left pulmonary (sixth aortic) arch; (*3*) right radix aortae; (*4*) dorsal aorta; (*5*) ligamentum aortae; (*6*) innominate arteries; (*7*) carotid (third aortic) arch; (*8a*) base of left internal carotid artery; (*8b*) cervico-intercostal artery; (*9*) subclavian arteries; (*10*) coracoid major; (*11*) coracoid minor; (*12*) ventral intercostal; (*13*) lateral intercostal; (*14*) pectoral; (*15*) coracoid and pectoral branches of pectoral artery; (*16*) axillary and pectoral branches of pectoral artery; (*17*) left basicervical; (*18a*) left vertebral; (*18b*) right vertebral; (*19*) right tracheal; (*20*) posterior dorsal intercostal; (*21*) anterior dorsal intercostals; (*22*) right subscapular; (*23*) right lateral superficial cervical; (*24*) right lymphatico-cutaneous; (*25*) left internal carotid (in hypapophysial canal); (*26*) ascending oesophageal; (*27*) myocervical; (*28*) right basicervical; (*29*) left tracheal; (*30*) thyroid-lymphatic; (*31*) thyroid gland and artery; (*32*) left posterior superficial cervical; (*33*) left anterior superficial cervical; (*34*) left ventral cutaneous; (*35*) left ventral anterior superficial cervical.

present in *Rhea americana* Linné, *Apteryx (australis) mantelli* Bartlett, and *Apteryx owenii* Gould. With regard to the arrangement of the carotids in the latter three species, the present writer's observations on the kiwi (*7*) and rhea are in agreement with the reported findings of Garrod (3), but the specimen of *Casuarius australis* which was examined by the present writer showed the left carotid artery alone as the vessel that entered the hypapophysial canal.

The following observations serve as an explanation of the diagram (Fig. 1) and the general distribution of the arteries of the neck and thorax.

Observations

In *Casuarius australis* the distal portion of the left embryonic sixth aortic arch remains as a ligament of fusion with the ligamentous vestige of the left radix aortae, to form the ligamentum aortae (*5*), while the corresponding portion of the right embryonic sixth aortic arch completely disappears in the mature bird. This probably results from atrophy of the right ligamentum Botalli and its ultimate fusion with the pulmonary artery proximally and the right radix aortae (*3*) distally.

As in other species of birds, the innominate arteries (*6*) give rise to the left (*7a*) and right (*7b*) carotid arches and the subclavian arteries (*9*). Each of the latter then gives rise to four arteries, the coracoid major (*10*), the intercostal (*12* and *13*), and two pectoral arteries (*14*). The intercostal artery divides to form ventral (*12*) and lateral (*13*) branches, and a small branch of the ventral intercostal serves as a coracoid minor (*11*) artery. The pectoral arteries are modified to supply the wings, pectoral muscles, and coracoid and scapular areas (*15* and *16*). In addition, the left subclavian artery sends off a basicervical artery (*17*) to supply the tissues at the base of the neck.

Anteriorly, each carotid arch (*7*) divides to give rise to a superior branch and an inferior branch. The left inferior artery gives rise to a small tracheal artery (*29*) before it divides to form the deep vertebral artery (*18a*) and the internal carotid artery (*25*) which enters the hypapophysial canal in the median line of the neck. The left superior artery sends branches to the thyroid gland (*31*) and connective tissues at the base of the neck (*30*), then bifurcates to form the ventral superficial (*35*) and lateral superficial (*33*) cervical arteries. The former sends off a branched myocervical artery (*27*), a ventral cutaneous artery (*34*), and several branches to the lymphatic glands and connective tissues along the left side of the neck. The lateral superficial cervical (*33*) gives rise to a posterior superficial cervical artery (*32*).

The superior branch of the right carotid arch gives rise to the posterior dorsal intercostal artery (*20*), several anterior intercostal arteries (*21*), and then becomes the right vertebral artery (*18b*). The inferior branch gives rise to the thyroid arteries (*31*) before sending off the large ascending oesophageal artery (*26*), right tracheal artery (*19*), right basicervical artery (*28*), lateral superficial cervical artery (*23*), and lymphatico-cutaneous artery (*24*). The

lateral superficial cervical gives off a small subscapular branch (*22*) before sending several branches to the muscles of the neck and surrounding connective tissues. The lymphatico-cutaneous artery supplies the lymphatic glands on the right side of the neck and ultimately terminates in the skin of the neck.

Discussion

It will be noted that, in the cassowary, the skin of the neck is supplied by vessels which arise in the cervical region, whereas in the kiwi, the skin is supplied by vessels which arise from the subclavian and pectoral arteries. Furthermore, the kiwi retains both the left ligamentum aortae and the right ligamentum Botalli (8), while the cassowary retains the ligamentum aortae alone.

Although these two species of birds differ in many respects, they do show two points of similarity. First, the left internal carotid artery alone enters the hypapophysial canal to pass forward to the head. Secondly, the axillary and pectoral arteries show remarkable adaptation to the reduction and modification of the wings and pectoral muscles in both species.

In general, however, the arterial arrangement in the cassowary is quite singular and largely unlike that observed in other species of birds which have thus far been examined by the writer. Representatives of more than 12 orders of birds have been studied, in part, to date, although but a few families have been reported (4 to 8). References to these arteries and their vestiges have been made also by Beddard (1), Bhaduri (2), and Stresemann (10).

Acknowledgments

The writer is indebted to Meems Bros. and Ward, Oceanside, Long Island, N.Y., for their kindness in making available the specimen used in this study, and to Dr. E. Horne Craigie, Department of Zoology, University of Toronto, Toronto, Ont., for his suggestions in the preparation of this paper.

References

1. BEDDARD, F. E. The structure and classification of birds. Longmans, Green, and Company, London. 1898.
2. BHADURI, J. L. A case of persistence of ductus caroticus in the Indian blue rock-pigeon *Columba livia intermedia* (Strick.) Anat. Anz. 88 : 178-182. 1939.
3. GARROD, A. H. On the carotid arteries of birds. Proc. Zool. Soc. London, 457-472. 1873.
4. GLENNY, F. H. A systematic study of the main arteries in the region of the heart—Aves I. Anat. Record, 76 : 371-380. 1940.
5. GLENNY, F. H. The main arteries in the region of the heart of three species of doves. Bull. Fan Memorial Inst. Biol., Zool. Ser., 10(4) : 271-278. 1940.
6. GLENNY, F. H. Presence of the ligamentum Botalli in the golden eagle *Aquila chrysaëtos* (L.), the red-tailed hawk *Buteo borealis borealis* (Gmelin) and the common pigeon *Columba livia* (L.). Ohio J. Sci. 41 : 46-49. 1941.
7. GLENNY, F. H. A systematic study of the main arteries in the region of the heart—Aves III, Fringillidae 1. Ohio J. Sci. 42 : 84-90. 1942.

8. GLENNY, F. H. Arteries in the heart region of the kiwi. Auk, 59(2) : 225-228. 1942.
9. HUXLEY, T. H. On the classification of birds; and on the taxonomic value of the modifications of certain of the cranial bones observed in that class. Proc. Zool. Soc. London, 415-472. 1867.
10. STRESEMANN, E. Aves. Handb. Zool. 72. 1927–1932.
11. WETMORE, A. A systematic classification of the birds of the world. Smithsonian Inst. Pub., Misc. Collections, 99 (7) : 1-11. 1940.

POLARIZATION AND PROGRESSION IN PAIRING

III. PACHYTENE OBSERVATIONS IN *NEODIPRION* (HYMENOPTERA)[1]

BY STANLEY G. SMITH[2]

Abstract

From an analysis of 72 nuclei at pachytene in three species of *Neodiprion* it is shown (1) that the rate of pairing is independent of chromosome size, (2) the nucleolar chromosomes are at no disadvantage in pairing, and (3) the incompletions observed are the result of an interruption by fixation of the process of zygotene pairing. It is concluded that there is no fixed relationship between the intimacy of telophase association and pachytene pairing, the degree of relational coiling at diplotene, and the distribution of chiasmata at metaphase.

On the basis of the positions of interlocks and the size of the loops containing them, Smith and Boothroyd (36) have presented evidence that pairing in *Trillium erectum* pollen mother cells starts in the region of the centromeres and progresses outwards towards the ends of the arms of the chromosomes. Following Bennett (2), Frankel (14) had reached a similar conclusion from an analysis of configuration types occurring in various *Fritillaria* species with and without localization of chiasmata. In addition, however, he found evidence of a secondary, distal, contact point which, since it acts later, is held subject to the action of a time limit. Similarly, Darlington (7) holds that procentric pairing is primarily operative in both diploid and tetraploid species of *Paris* having localized chiasmata and that secondary contact points are situated in other regions of the chromosomes. The exact position in which pairing begins is considered to depend on the shape and size of the chromosome. Barber (1) finds in *Uvularia* that the reduction in chiasma frequency, induced by cold treatment, is differential in spatial distribution, the middle regions of long arms being more readily affected. He concludes that procentric and proterminal contact points are normally operative.

Darlington considers that his evidence implies a regular orientation of the chromosomes in the leptotene stage of the normal nucleus as the basis of regular co-ordination of pairing. Barber interprets his findings as showing that localization of pairing depends on (*a*) proximity of certain regions of the chromosomes, which is determined by a persistence of the telophase arrangement from the last premeiotic mitosis, and (*b*) a greater freedom of movement of short chromosomes or of the ends of long ones. Smith and Boothroyd hold that the centric and adjacent regions of homologues have an initial advantage in pairing as a result of the chromosomes being polarized since the last premeiotic anaphase disjunction, but recognized that the ends of the chromosomes, if not already associated, will also be brought together

[1] *Manuscript received August 6, 1942.*

Contribution No. 2194 from the Division of Entomology, Science Service, Dominion Department of Agriculture, Ottawa, Canada.

[2] *Presently stationed at, and Research Associate in McGill University, Montreal, Que.*

when the nuclear membrane is reformed, as is seen in a comparison of mitotic telophase and prophase (Rabl's (30) orientation). From a comparison of Diptera with other animals and plants, Smith (34) has given reasons for concluding that the pairing of homologues, consummated at pachytene, is initiated at the anaphase of the last premeiotic division.

Both Darlington and Barber assume that there is a time factor in pairing and that short chromosomes and short chromosome arms start pairing earlier than long ones and complete their pairing earlier. This they hold is the result of a greater freedom of movement in pairing *at zygotene*. Smith, on the other hand, concludes that short chromosomes and short chromosome arms can become more completely associated at the *last premeiotic telophase* as a direct result of their shortness and closer congression at the anaphase pole. Darlington's and Barber's assumption is based on interpretation of indirect evidence; certain data collected during a study of sawfly oocytes supply direct evidence on this point.

With further regard to the time factor as a limitation to pairing, Darlington (4, 5, 6) has stated that static bodies such as nucleoli delay the pairing of the chromosomes to which they are attached, and thus interfere with chiasma formation. He considers that this indicates why ". . . large nucleoli are as a rule situated near the ends . . ."; "Evidently they would hinder meiotic pairing too much if they lay in the middle of the chromosomes." This conclusion regarding interference is based on somewhat meagre evidence and is not supported by McClintock's (27) drawings of *Zea mays*, nor by Ernst's (8, 9) observations of pachytene in *Antirrhinum*. The analysis of the present data will go further in determining the general validity of this hypothesis. This is particularly important since the sex chromosome pair in many Diptera, including *Drosophila*, carries the nucleolus, and delay in pairing in localized regions will therefore affect the genetic map of the X-chromosome.

Finally, Darlington (4) reports for species of *Fritillaria* with a normal distribution of chiasmata that although "It has always been supposed that pairing in such species was complete . . .", he now rarely finds a nucleus with complete pairing. He attributes the earlier opinion to the fact that "An occasional unpaired segment would be taken as indicating that the nucleus was still in an active zygotene condition."

From the observations here presented it is intended to test the general applicability of three assumptions (i) rapidity of pairing is dependent on the size of the units involved, (ii) nucleoli tether the chromosomes to which they are attached and thereby delay their pairing, and (iii) incomplete pairing of chromosomes is a characteristic even of species with a normal chiasma distribution.

Material

Females of three species of the hymenopteran genus *Neodiprion*, namely, *N. dubiosus*, *N. lecontei*, and *N. swainei*, have been studied at pachytene. Slides were prepared by the Feulgen squash method, after fixation for about

10 min. in a modification of Kahle's fluid (35). Light green was used to stain the nucleolus. All pachytene nuclei were drawn with the aid of a camera lucida using a Zeiss 1/12 homogene immersion objective and a 20× ocular; the single somatic metaphase illustrated was drawn using a Zeiss 1.5 mm., 1.3 N.A. objective.

Observations

The diploid number of chromosomes in females of all three species is 14. This is the number previously found by Smith (33) in *N. sertifer*. The centromeres are approximately median in all pairs of homologues except one of the three of medium size, so that in each of six pairs the arms are of almost equal length (see Fig. 1, the complement in a male of *N. lecontei*).

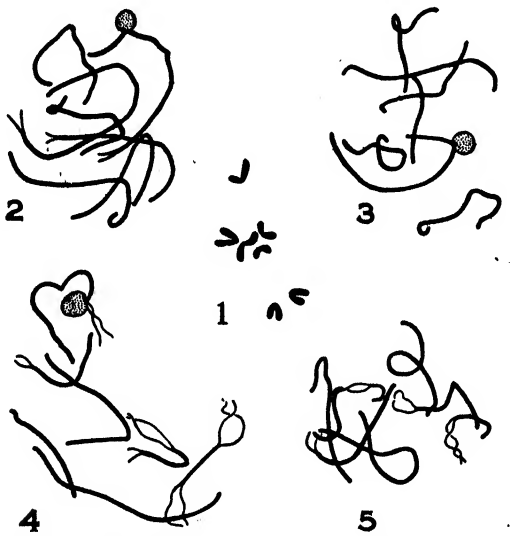


FIG. 1. *The somatic chromosomes of N. lecontei*, ♂. (×*2200*).
FIGS. 2 TO 5. *Pachytene chromosomes of females.* (×*1800*).
FIG. 2. *Polarization and distal incompletions in N. lecontei.*
FIG. 3. *Complete pairing in N. dubiosus.*
FIG. 4. *Distal incompletions in N. lecontei showing especially that of the nucleolar chromosome.*
FIG. 5. *Distal and one interstitial incompletion in N. swainei.*

As in other species of the Diprionidae (33), the arrangement of the seven bivalents at pachytene shows clear evidence of centric polarization (Fig. 2), although this is frequently more or less obscured by excessive pressure in making the preparation. In all a total of 72 whole complements have been drawn at pachytene from 17 females belonging to the three species (Table I). The association between the homologues, if complete, is then so close as to make the bivalents appear like single, thick threads (Fig. 3). The seven pairs are of different lengths. In Table I their individual lengths are expressed

TABLE I

THE DISTRIBUTION OF UNPAIRED REGIONS AMONG PACHYTENE BIVALENTS IN VARIOUS SIZE CLASSES

Species	No. ♀♀ examined	No. cells drawn	Length of bivalents expressed as percentage of whole complement							Total distals
N. lecontei	7	33	18.9 7*D**	17.3 7*D* + 1*I*	15.9 3*D*	14.7 2*D*	12.8 7*D**	11.3 10*D* + 1*I*	9.1 7*D*	41*D*
N. dubiosus	5	22	19.4 1*D*	17.2 —	15.9 3*D*	14.8 2*D*	13.4 2*D*	11.1 1*D*	8.2 2*D*	11*D*
N. swainei	5	17	19.5 —	17.1 —	15.7 —	14.6 2*D*	13.1 1*D*	11.3 1*D*	8.8 4*D*	8*D*
Mean length Total distals	17	72	19.3 7*D*	17.2 7*D*	15.8 6*D*	14.7 6*D*	13.1 9*D*	11.2 12*D*	8.7 13*D*	60*D*
Expected on random basis			8.6	8.6	8.6	8.6	8.6	8.6	8.6	$\chi^2 = 5.80$
Expected on length basis			11.6	10.3	9.5	8.8	7.9	6.7	5.2	$\chi^2 = 28.40$

*D = distal incompletion; I = interstitial incompletion; * = 1 distal interlock omitted from totals, see text.*

as percentages of the whole complement; they are arranged in decreasing order. In preparation the chromosomes were squashed flat so that there was very little or no optical foreshortening. The mean percentage lengths have been given in preference to absolute lengths because of variation between cells resulting from slight differences in stage and from the differential effect of the fixative.

Within the species the minimum length difference separating bivalents in the seven successive size classes was always at least 1.1 per cent, with the longest bivalent more than twice the length of the shortest. Between species the mean lengths of the bivalents compared in order of size never differed by more than 0.9 per cent. The three species therefore have complements sufficiently similar to warrant grouping them.

In 64 out of the total of 504 bivalents drawn and measured, pairing was incomplete (Figs. 2, 4, and 5) and in every case but two the incompletions were situated at the distal ends of the bivalents. Of these two interstitial incompletions, one was the result of "false" interlocking, its chromosomes having encircled those of another bivalent. The reason for the second (Fig. 5) could not be determined. Two of the distal incompletions were due to potential interlocking and therefore, like the previous two, will be omitted from further consideration. Thus there remains a total of 60 incompletions, 41 in *N. lecontei*, 11 in *N. dubiosus*, and 8 in *N. swainei*, to distribute between the seven size classes of bivalents.

Out of the total of 72 nuclei drawn, incomplete pairing occurred in only 30; one of these, in *N. lecontei*, was due to interlocking and may therefore, from

the present point of view, be considered as completely paired. Pairing was thus complete in 43 nuclei, 15 in *N. lecontei*, 16 in *N. dubiosus*, and 12 in *N. swainei*. Considering the frequency of incompletions in individual females, nine of the 17 examined had only cells with complete pairing, seven had cells with both complete and incomplete pairing and one had only partial pairing. In this single female, however, only two cells were suitable for detailed analysis. The occurrence in all individuals except one of cells in which the chromosomes are fully associated suggests that the observed incompletions were the result of fixing the ovaries while the nuclei were still undergoing active pairing. This is shown also (Table II) by the fact that as the number of incompletions per cell increases, from zero to six—the maximum number observed—the frequency of cells showing these numbers of incompletions steadily decreases from 43 to one. It might here be mentioned that in these 72 nuclei and in about three times as many more (which for various reasons were unsuitable for exact measurement) there was absolutely no evidence of structural hybridity.

TABLE II

THE FREQUENCY OF CELLS WITH DISTAL INCOMPLETIONS IN DIFFERENT SPECIES

Species		Number of incompletions per cell								
		0	1	2	3	4	5	6	7	Total
N. lecontei	No. of cells	15	8**	3	4*	1	1	1**	0	33
N. dubiosus	No. of cells	16	3	2	0	1	0	0	0	22
N. swainei	No. of cells	12	4	0	0	1	0	0	0	17
	Total	43	15	5	4	3	1	1	0	72

*, ** *Omitting one interstitial and one distal incompletion respectively, see text.*

In Table I the distribution of the unpaired ends among the various types of bivalents is given and compared by means of the χ^2 test of goodness of fit with expectation, first, on a random basis and, second, on the assumption that the time required for complete pairing is directly proportional to length. From the first comparison $\chi^2 = 5.80$, which, with $N = 6$, gives $P = 0.50$; from the second, $\chi^2 = 28.40$ and $P = < 0.01$. It is therefore evident that the short chromosomes in these species complete their pairing no more readily than the long chromosomes. Since, as already stated, all the chromosomes, with the exception of one in the three middle size classes, are mediocentric, a similar conclusion holds with regard to the chromosome arms.

In 26 of the 30 cells with partial pairing, the nucleolar chromosomes could be identified by their association with the nucleolus. The nucleolar constriction at mitotic metaphase is situated about half-way along an arm of either the longest or second longest chromosome (Fig. 1). In the 26 cells there was a total of 52 incompletions, 51 distal, and one interstitial, omitting

those resulting from interlocking. Of the unpaired regions, 34, including the single interstitial, occurred in the bivalents of cells in which the nucleolar chromosomes were completely associated, three were situated on this chromosome pair in cells in which the remaining chromosomes were fully paired, and five involved the nucleolar pair in cells having 10 other incompletions (see Fig. 4). In short, the nucleolar chromosomes were incompletely associated on eight occasions, the other chromosomes on 44.*

Discussion

Darlington (4) has assumed that the rarity of nuclei with complete pairing in species of *Fritillaria* "with a normal distribution of chiasmata" is the result of the operation of a time limit which even in these species is too early to allow full association; he does not attribute them to an interruption by fixation of the process of zygotene pairing. Even if zygotene is completed, the possibility nevertheless remains for plants and especially such plants as *Fritillaria*, which reproduce largely by vegetative means, that the failure to pair completely may be due to structural hybridity. Inversions have been reported in nine species of this genus by Frankel (13, 14), Bennett (2), and Darlington (see 13). In *Triticum vulgare*, which is almost entirely self-fertilized and which reproduces only sexually, normal varieties show complete association at pachytene as Dark (3) has shown for a single nucleus of *Bellevallia*, while heterozygous deficiency mutations show unpaired loops at this stage (Huskins and Smith, manuscript in preparation) as McClintock (26) has shown in *Zea mays*. The complete pairing observed herein implies a total absence of structural hybridity in the Hymenoptera studied; this is presumably the result of the haploid constitution of the males; it follows that in the absence of the buffering effect of a second set of homologues selective elimination would come immediately into play (17).

That complete association of chromosomes at pachytene is the rule rather than the exception was the conclusion reached by many of the early workers on animals (cf. 46). Complete association of the chromosomes is found in other materials now being studied such as *Plethedon*, various Orthoptera, and man (material in the Department of Genetics, McGill University). It seems evident from McClintock's (26, 27) work on *Zea mays*, Levan's (see later) and Sax and Sax's (32) on *Allium*, and Ernst's (8, 9) on *Antirrhinum majus* that the same conclusion applies to plants. Hence the occurrence of unpaired regions appears to be the result of an interruption by fixation of the active process of pairing or of a lack of linear homology, rather than proof of the action of a time limit for pairing.

As was stated under the heading "Observations", in the 26 cells having incomplete pairing and in which the nucleolar chromosomes could be identified, these chromosomes were observed to be either completely or incompletely

** Since the foregoing was sent to press another species of sawfly, Gilpinia frutetorum, has been similarly analysed. Thirty-six nuclei at pachytene have been drawn and measured with results that agree in all respects with those obtained from the Neodiprion species.*

paired, while certain of the other chromosomes in the same cells were also either partially unpaired in the former or completely or partially paired in the latter. The nucleolar chromosomes were incompletely associated on eight occasions, the other chromosomes on 44. If incompletion of pairing is a random phenomenon the proportion expected is about 7.5 to 44.5—clearly the nucleolar chromosomes in these *Neodiprion* species are at no disadvantage during pairing relative to the other chromosomes. That they should be, if the association between homologues were initiated at zygotene, when the chromosomes are fine and drawn out, is a logical expectation. If, however, an active side by side association occurs by telophase of the last premeiotic division, as the writer believes, when no chromosome is handicapped in movement by an attached nucleolus, there should be no delay in the pairing of the nucleolar chromosomes.

Ernst (8) finds in *Antirrhinum majus* that at zygotene 88.5% of the cells have only one nucleolus. The nucleolar chromosomes, which are terminally attached to the nucleolus, were sometimes completely paired and sometimes separated on either side of the nucleolus. In the latter case they were seen to become united soon after leaving the nucleolus. In the remaining 11.5% the two nucleoli were in most cases relatively close to one another and the nucleolar chromosomes were associated in most of the proximal region. Whether in these rare cases the "static" nucleoli were actually hindering pairing or not is difficult to say because, if pairing is initiated centrically in *Antirrhinum*, as is probable, and progresses outwards, the unpaired regions may have been the result of fixing too early and thus interrupting the pairing process.

With regard to the third assumption, that the rapidity with which pachytene is completed is a function of chromosome length, the evidence appears strongly contrary.

Smith (34) has tabulated evidence from the literature showing that in most phyla of the Metazoa homologous chromosomes associate in pairs by the telophase of the last premeiotic mitosis. The observations of Stevens (38) and Foot and Strobell (12) on *Sagitta bipunctata* and *Allolobophora foetida* respectively leave no doubt that in these the pairing at telophase is intimate throughout the length of the chromosomes. Sutton (42), however, has demonstrated that while short chromosomes and short chromosome arms in the lubber grasshopper, *Brachystola magna*, associate completely at the last premeiotic anaphase, long chromosomes, many times the length of the former, associate only in their centric regions. Such an organism would be especially vulnerable to the action of a time limit for pachytene pairing.

In neither *Allolobophora* nor *Sagitta* are the chromosomes of the haploid complement of markedly different size (as they are in the orthopteran) being, in fact, somewhat similar in size range to those of the *Neodiprion* species studied here. The long chromosomes and the long chromosome arms in the lubber grasshopper are confined at telophase within compartments which open into a common chamber situated at the polar end of the nucleus. In these long chromosome arms association is therefore restricted to the centric

regions. The short chromosomes, however, are of insufficient length to allow their projection from the common chamber and hence they associate throughout their entire length. In other grasshoppers, with chromosomes of the same relative proportions, the side by side association of homologues, often initiated in the early gonial divisions (45), is completed by the end of the last premeiotic division.

Despite the fact that telophase pairing is localized at the centric ends of the chromosomes in *Brachystola*, pachytene association is nevertheless complete and chiasmata are formed apparently at random along the bivalents, although by diakinesis they are largely situated at or near the two ends of the bivalent. Foot and Strobell's (11, 12) photomicrographs of *Allolobophora* show that apparently complete pachytene pairing is similarly followed by chiasmata largely terminal at metaphase, even though in this case the premeiotic association was demonstrably complete. Unfortunately, Stevens' illustrations of the first metaphase chromosomes of *Sagitta bipunctata* fail to supply conclusive evidence of the relationship between the distribution of chiasmata and the complete pairing observed at telophase (38) and pachytene (39).

Pachytene pairing is not a simple continuation of telophase side by side association; this has to all intents and purposes been recognized by Fabergé (10). He points out that there is a difference between the initial pairing movements of leptotene–zygotene (the telophase association of the present author) and the close, or contact pairing of pachytene. He enumerates several points that he considers evidence against the general belief that the polarization of the ends is a necessary preliminary to normal pairing, for example, first, there is often no polarization, or it is variable in degree; second, in the bouquet the ends are often some distance apart; and, third, zipper pairing will not work in structural hybrids. Further, he considers the same objectinns apply to pairing being initiated at the centromere. It is clear that none of these objections is legitimate if side by side association arises at telophase, as has earlier been argued by the present author (34).

In the Diptera it has been conclusively proved that somatic pairing at prophase and metaphase is derived from the side by side association of homologues at the previous anaphase, and Hance (15) has shown that the prophase association is in the form of relational coiling. Similarly in the Diptera, it has been shown that the parallel association between homologues at the last premeiotic anaphase is supplanted in meiosis by relational coiling (29, 40, 41, 44, etc.). In other organisms this same relational coiling between chromosomes at meiosis replaces the relational coiling earlier apparent between chromatids at mitosis. Sparrow, Huskins, and Wilson (37) have given reasons for believing that somatic relational coiling is a carry-over from the previous division of the "plectonemic coiling" of the half chromatids. They deny that it arises as the result of an active twisting of the chromatids around one another as Darlington (4, 5) contends. Whether this is so or not, it is clearly recognized by Huskins (17) that the relational coiling *between chromo-*

somes seen at diplotene must result from an active twisting, and not from a carry-over. He considers it unproved that diplotene twists are the residuum after chiasma formation, pointing out that they might even be caused, not eliminated, by the chiasmata. However, the evidence available, meagre though it is, favours Darlington's interpretation, since relational coiling is demonstrably present both in the absence of crossing-over (male *Drosophila*) and in the absence of chiasma formation as shown by Richardson (31) on "asynaptic" *Crepis*; and Levan (24) in "asynaptic" triploid *Allium amplectens*.

From his extensive studies on the genus *Allium*, Levan has made valuable contributions to the knowledge of the relationships between pairing, relational coiling, and the position of chiasmata. Among the species that he has examined that supply evidence on these relationships are the eight listed in Table III.

TABLE III

THE RELATIONSHIPS BETWEEN PACHYTENE PAIRING, RELATIONAL COILING, AND CHIASMA POSITION IN *Allium*

Species	Diploid chromosome number	Degree of pachytene pairing	Extent of relational coiling	Distribution of chiasmata at metaphase	Reference
A. ammophylum	16	Complete	Complete	Random	Levan (23)
A. rosenbachianum	16	?	Complete	Random	Levan (23)
A. amplectens	28	Complete*	Complete	Random	Levan (24)
A. macranthum	28	Complete**	?	Random	Levan (22)
A. fistulosum	16	Complete	Complete	Localized	Levan (21)
A. farreri	16	Complete	Complete	Localized	Levan (23)
A. porrum	32	*Ca.* complete	Complete	Localized	Levan (25)
A. amplectens	21	Complete*	Complete	Zero	Levan (24)

* *Pairing complete except for exchanges of partner in multivalents.*
** *Pairing complete except for interlocking.*

From this table it will be seen that regardless of whether the chiasmata at metaphase are distributed at random, localized in regions close to the centromeres, or entirely absent, pachytene association is nevertheless complete or nearly so. Regarding the occasional unpaired portions, Levan points out that they result from (1) visible interlocking (*A. macranthum*), (2) exchanges of partner between the constituents of quadrivalents, or (3) structural hybridity as shown by dissimilarities in form and number of chromomeres (*A. amplectens*, $4n$), and that at mid-pachytene they were not more frequently towards the ends of the bivalents (*A. porrum*).

Despite the subsequent variation in the position of chiasmata and also their absence in triploid *A. amplectens*, relational coiling was uniformly present

at diplotene[1]. It is thus evident that in *Allium* the position of chiasmata at metaphase bears no fixed relation to the extent to which pairing has proceeded at pachytene; the type of pairing at pachytene is not to be inferred from the distribution of chiasmata at metaphase. Bennett's (2) statement, "There is evidence, however, from the occasional persistence of relational coiling, at diakinesis, in arms of chromosomes having no chiasmata, that such arms have been unpaired or intermittently paired at pachytene", obviously ignores Levan's earlier evidence to the contrary.

Darlington (6) recognizes the fallacy of this assumption in that the chiasma frequency varies in different clones of *Fritillaria imperialis* despite their all having "nearly complete pairing". Clearly his precocity theory of meiosis and the principle of one-by-one attraction demand, first, that paired chromosomes are single at the time of pairing, and, second, that their division upsets this attraction; hence, he concludes, "torsion, or whatever else is responsible for their crossing-over potential" must vary. This Darlington considers to be the third prime variable of meiosis, the other two being the point of contact or the position in which pairing is initiated, and the time limit. It alone appears to be the variable the existence of which is positively established. Although it is almost certain that a random distribution of chiasmata at metaphase is a consequence of complete pairing at pachytene, it is doubtful whether any other position at metaphase bears any direct relation to the contact point and the postulated time limit, Mather (28) to the contrary. In *Drosophila melanogaster* a comparison of the genetic and cytological maps indicates that the chiasma frequency is lower in the centric regions than elsewhere. On the other hand, in those species of *Fritillaria* and *Mecostethus* having extreme centric localization, the chiasmata are concentrated around the centromere. Yet in all three, pairing starts in the neighbourhood of the centromere. Whatever it is that causes crossing-over must be responsible for this differential distribution, just as it must determine the different behaviour of the various *Allium* species.

In view of the conclusions reached independently by Fabergé (10) and Smith (34), one may expect that once the side by side association of homologues is established by telophase, the chromosomes will be in a position for the intimate pairing of the ensuing zygotene–pachytene to commence. Clearly, whether it will start from one or a number of points will depend on the degree to which telophase association had proceeded prior to demobilization at the resting stage. Possibly in some species showing restriction in chiasma distribution, such as *Fritillaria meleagris* and *Mecostethus grossus*, telophase association may be only partial and hence centrically localized; zygotene

[1] *In A. fistulosum and A. farreri, Levan attributed the more distal loops seen at diplotene to opening out between successive chiasmata. It seems evident now that these loops were the result of relational coiling, as Levan now interprets them in A. porrum. White (43), however, still considers them to be chiasmata freely distributed at diplotene, which have moved towards the centromere by metaphase, as reported by Hearne and Huskins (16) for Melanoplus femur-rubrum. Since chiasmata at diplotene are in unspiralized and somewhat widely spaced threads, which, however, become closely associated and spiralized by metaphase, it seems probable that the supposed movement is an optical effect due to decreasing parallax (18).*

pairing would then be hindered and, if prophase is of short duration, a time limit might come into play so that the unpaired regions divide and thereby interrupt pairing, as shown for *F. meleagris* by Huskins and Smith (19). In others, e.g., *Allium fistulosum*, etc., a similar delay might result in a lowering of the crossing-over potential (6) in distal regions but, if there were no interruption by division, pairing should be completed and relational coiling should be seen at diplotene. In yet others, such as "asynaptic" triploid *Allium*, the crossing-over potential might be reduced throughout the chromosomes below the necessary minimum. Terminal localization might then result not necessarily from distal contact but from the loss of crossing-over potential being restricted to the centric regions. Genetic data imply a variation in crossing-over potential in different regions of the chromosomes of *D. melanogaster*, yet it is in this species of all organisms that it is certain that association is intimate throughout the length of the chromosomes at somatic telophase and presumably at that of the last gonial division. It is therefore concluded that there is no fixed relationship between the intimacy of telophase association and pachytene pairing, the degree of diplotene relational coiling, and the distribution of metaphase chiasmata.

Acknowledgment

It is a pleasure to express my gratitude to Professor C. L. Huskins and Dr. H. G. Sander for reading and criticizing the manuscript.

References

1. BARBER, H. N. Chromosome behaviour in *Uvularia*. J. Genetics, 42 (1 and 2) : 223-257. 1941.
2. BENNETT, E. S. The origin and behaviour of chiasmata. XIV. *Fritillaria chitralensis*. Cytologia, 8 (3 and 4) : 443-451. 1938.
3. DARK, S. O. S. Chromosome studies in the Scilleae. II. J. Genetics, 29 (1) : 85-98. 1934.
4. DARLINGTON, C. D. The internal mechanics of the chromosomes. II. Prophase pairing at meiosis in *Fritillaria*. Proc. Roy. Soc. (London) B, 118 : 59-73. 1935.
5. DARLINGTON, C. D. The external mechanics of the chromosomes. II. Meiosis in diploids. Proc. Roy. Soc. (London) B, 121 : 273-289. 1936.
6. DARLINGTON, C. D. The prime variables of meiosis. Biol. Rev. Cambridge Phil. Soc. 15 (3) : 307-322. 1940.
7. DARLINGTON, C. D. Polyploidy, crossing-over, and heterochromatin in *Paris*. Ann. Botany (n.s.), 5 (18) : 203-216. 1941.
8. ERNST, H. Meiosis und Crossing over. Zytologische und genetische Untersuchungen an *Antirrhinum majus* L. Z. Botan. 33 (6 and 7) : 241-294. 1938.
9. ERNST, H. Meiosis und Crossing over. Zytogenetische Untersuchungen an *Antirrhinum majus* L. Z. Botan. 34 (2) : 81-111. 1939.
10. FABERGÉ, A. C. Homologous chromosome pairing: the physical problem. J. Genetics, 43 (1 and 2) : 121-144. 1942.
11. FOOT, K. and STROBELL, E. C. Prophases and metaphases of the first maturation spindle of *Allolobophora foetida*. Am. J. Anat. 4 : 199-243. 1905.
12. FOOT, K. and STROBELL, E. C. Pseudo-reduction in the oögenesis of *Allolobophora foetida*. Arch. Zellforsch. 5 : 149-165. 1910.
13. FRANKEL, O. H. Inversions in *Fritillaria*. J. Genetics, 34 (3) : 447-462. 1937.
14. FRANKEL, O. H. The causal sequence of meiosis. I. Chiasma formation and the order of pairing in *Fritillaria*. J. Genetics, 41 (1) : 9-34. 1940.
15. HANCE, R. T. The somatic mitoses of the mosquito *Culex pipiens*. J. Morphol. 28 : 579-591. 1917.

16. HEARNE, E. M. and HUSKINS, C. L. Chromosome pairing in *Melanoplus femur-rubrum*. Cytologia, 6 (2 and 3) : 123-147. 1935.
17. HUSKINS, C. L. Polyploidy and mutations. Am. Naturalist, 75 (759) : 329-344. 1941.
18. HUSKINS, C. L. and NEWCOMBE, H. B. An analysis of chiasma pairs showing chromatid interference in *Trillium erectum* L. Genetics, 26 : 101-127. 1941.
19. HUSKINS, C. L. and SMITH, S. G. Chromosome division and pairing in *Fritillaria meleagris*: the mechanism of meiosis. J. Genetics, 28 (3) : 397-406. 1934.
20. HUSKINS, C. L. and SMITH, S. G. Chromosome mutations in polyploid *Avena* and *Triticum*. II. (In manuscript).
21. LEVAN, A. Cytological studies in *Allium*. IV. *Allium fistulosum*. Svensk Botan. Tid. 27 (2) : 211-232. 1933.
22. LEVAN, A. Cytological studies in *Allium*. V. *Allium macranthum*. Hereditas, 18 : 349-359. 1933.
23. LEVAN, A. Cytological studies in *Allium*. VI. The chromosome morphology of some diploid species of *Allium*. Hereditas, 20 : 289-330. 1935.
24. LEVAN, A. The cytology of *Allium amplectens* and the occurrence in nature of its asynapsis. Hereditas, 26 : 353-394. 1940.
25. LEVAN, A. Meiosis in *Allium porrum*, a tetraploid species with chiasma localization. Hereditas, 26 : 454-462. 1940.
26. MCCLINTOCK, B. Cytological observations of deficiencies involving known genes, translocations, and an inversion in *Zea mays*. Missouri Agr. Expt. Sta. Research Bull. 163. 1931.
27. MCCLINTOCK, B. The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Z. Zellforsch. mikroskop. Anat. 21 (2) : 294-328. 1934.
28. MATHER, K. The determination of position in crossing-over. III. The evidence of metaphase chiasmata. J. Genetics, 39 (2) : 205-223. 1940.
29. METZ, C. W. and NONIDEZ, J. F. Spermatogenesis in the fly, *Asilus sericeus* Say. J. Exptl. Zool. 32 : 165-185. 1921.
30. RABL, C. Über Zelltheilung. Morphol. Jahrb. 10 : 214-330. 1885.
31. RICHARDSON, M. M. Meiosis in *Crepis*. II. Failure of pairing in *Crepis capillaris* (L.) Wallr. J. Genetics, 31 (1) : 119-143. 1935.
32. SAX, H. J. and SAX, K. Chromosome structure and behavior in mitosis and meiosis. J. Arnold Arboretum, 16 : 423-439. 1935.
33. SMITH, S. G. A new form of spruce sawfly identified by means of its cytology and parthenogenesis. Sci. Agr. 21 (5) : 245-305. 1941.
34. SMITH, S. G. Polarization and progression in pairing. II. Premeiotic orientation and the initiation of pairing. Can. J. Research, D, 20 (8) : 221-229. 1942.
35. SMITH, S. G. Techniques for the study of insect chromosomes. Can. Entomol. (In press). 1942.
36. SMITH, S. G. and BOOTHROYD, E. R. Polarization and progression in pairing. I. Interlocking of bivalents in *Trillium erectum* L. Can. J. Research, C, 20 (7) : 358-388. 1942.
37. SPARROW, A. H., HUSKINS, C. L., and WILSON, G. B. Studies on the chromosome spiralization cycle in *Trillium*. Can. J. Research, C, 19 (9) : 323-350. 1941.
38. STEVENS, N. M. On the ovogenesis and spermatogenesis of *Sagitta bipunctata*. Zool. Jahrb. Abt. II, 18 : 227-240. 1903.
39. STEVENS, N. M. Further studies on the ovogenesis of *Sagitta*. Zool. Jahrb. Abt. II, 21 : 243-252. 1905.
40. STEVENS, N. M. A study of the germ cells of certain Diptera, etc. J. Exptl. Zool. 5 : 359-374. 1908.
41. STEVENS, N. M. The chromosomes in the germ-cells of *Culex*. J. Exptl. Zool. 8 : 207-225. 1910.
42. SUTTON, W. S. On the morphology of the chromosome group in *Brachystola magna*. Biol. Bull. 4 : 24-39. 1902.
43. WHITE, M. J. D. Chiasma-localisation in *Mecostethus grossus* L. and *Metrioptera brachyptera* L. (Orthoptera). Z. Zellforsch. mikroskop. Anat. 24 (1) : 128-135. 1936.
44. WHITING, P. W. The chromosomes of the common house mosquito, *Culex pipiens* L. J. Morphol. 28 : 523-577. 1917.
45. WILSON, E. B. Studies on chromosomes. VIII. Observations on the maturation-phenomena in certain Hemiptera, etc. J. Exptl. Zool. 13 : 345-450. 1912.
46. WILSON, E. B. The cell in development and heredity. 3rd ed. The Macmillan Company, New York. 1925.

STUDIES ON FACTORS INFLUENCING THE HEALTH OF PIGS

I. THE RELATIONSHIP OF BLOOD HAEMOGLOBIN CONCENTRATIONS TO RATE OF GAIN IN SUCKLING PIGS[1]

By W. E. Swales[2], E. W. Crampton[3], G. C. Ashton[4], and L. Choquette[5]

Abstract

The trends of blood haemoglobin concentrations in suckling pigs in a herd in which clinical anaemia does not occur have been studied and compared to rates of gain in body weight.

Ferrous sulphate and reduced iron were then used to keep the haemoglobin concentrations at higher levels, and statistical analysis of the observations showed a significant difference in body weight at weaning time between the treated and control groups.

The results indicate that pigs should be provided with a source of iron within a day or two after birth in order to ensure normal development and, possibly, resistance to disease.

Work on this and other possible factors in disease resistance is being continued.

Introduction

The importance of nutritional anaemia as a cause of loss in suckling pigs that are kept indoors has resulted in the collection by many workers of a large number of data on the nature, prevention, and treatment of the disease. In Canada a high percentage of litters are, of necessity, farrowed indoors and many of them have no access to soil and vegetation during the first few weeks of life. The precipitous drop in haemoglobin from the birth level, which approximates that of an adult animal, to one bordering on clinical anaemia occurs during the first 10 days of life. Unless this drop is checked by some form of therapy a serious disease often occurs, and it is now generally recognized that clinical anaemia predisposes to other pathological conditions. Hamilton, Hunt, and Carroll (4) define clinical anaemia in pigs as the condition that results when the blood haemoglobin concentration reaches a value of 3.5 gm. or less per 100 cc. of blood. As the haemoglobin level in the newborn pig approximates 11.5 gm.%, the intermediate stage between the birth and anaemia levels is relatively wide.

The work of Schofield (7) in Canada resulted in steps being taken to prevent clinical anaemia and only uninformed stock owners now fail to give attention to preventive measures for confined litters. However, many farmers do not

[1] *Manuscript received June 6, 1942.*

Contribution from the Division of Animal Pathology, Science Service, Dominion Department of Agriculture, the Department of Animal Nutrition, McGill University, Macdonald College, and the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Associate Animal Pathologist, Science Service, Dominion Department of Agriculture (Institute of Parasitology, Macdonald College).*

[3] *Professor of Animal Nutrition, McGill University, Macdonald College.*

[4] *Assistant in Animal Experimentation, Macdonald College.*

[5] *Research Assistant, Institute of Parasitology, McGill University, Macdonald College.*

supply any form of iron supplement until the pigs are several days old, at which time the haemoglobin may have dropped to less than 75% of the birth level. For this reason the effect on growth and resistance to disease of such subclinical anaemias is still open to question. According to Moe, Craft, and Thompson (6), groups of pigs that received various iron supplements and those that received no supplement but did not develop clinical anaemia, made similar gains in weight; however, the groups receiving iron were significantly heavier at weaning age than the untreated control group.

The herd of pigs that is used for research in animal nutrition at Macdonald College rarely suffers losses from anaemia. This has been ascribed to the dietary regime, which includes an abundance of iron (0.5% of ferric oxide in the supplement) in the ration of the pregnant and nursing sows and, theoretically, ensures an adequate storage in the new-born pig. In addition, the faeces of the sow has a high iron content and this, spread around the pens, is available for the suckling pigs. Because no clinical anaemia had occurred, no direct dosing of pigs had been practiced up to 1941. Although the records of growth of the majority of young pigs had been very satisfactory, an examination of the data for 1940 showed that a flattening of the growth curve between the ages of 10 and 21 days occurred. As this period appeared to coincide with a period of susceptibility to Ascaris infection in pigs in other parts of the Dominion (as found by Choquette (2)), in a preliminary survey, it was decided to investigate the blood haemoglobin levels in the litters born during 1941.

Procedure

Eight litters, comprising 61 pigs born during January, February, and March, 1941, were used for preliminary measurements of blood haemoglobin levels. The measurements were made by the Dare haemoglobinometer; one person took all the readings after it was determined by checking on a photoelectric colorimeter that his interpretations of the matching colours were relatively

TABLE I

OBSERVATIONS ON BLOOD HAEMOGLOBIN AND GAIN IN BODY WEIGHT OF 61 PIGS BORN IN JANUARY, 1941

Age (days)	Weight (lb.) and standard deviations	Weight (lb.)*	Daily gain (lb.)**	Haemoglobin (gm.%) and standard deviations
1	2.9 ± 0.3	2.8	—	9.9 ± 1.7
8	6.6 ± 0.9	6.8	0.50	6.7 ± 1.2
15	10.0 ± 1.3	10.1	0.47	5.7 ± 1.2
22	13.2 ± 1.9	12.9	0.41	5.9 ± 1.5
29	15.6 ± 2.4	15.6	0.39	6.5 ± 1.7
36	18.5 ± 2.9	18.4	0.40	7.6 ± 2.2
43	21.4 ± 3.1	21.6	0.45	8.5 ± 2.1
50	25.4 ± 4.1	25.3	0.53	10.5 ± 1.9

* *A third degree curve fitted by the summation method of fitting polynomials (Fisher (3)).*

** *These values are the differences between the polynomials divided by the number of days between times of weighing.*

accurate. The results of these observations, together with data on the weights and rates of gain, are shown in Table I and Fig. 1. In Table I the standard deviations shown indicate the normal variation between individual pigs within litters, the difference between sows having been removed*.

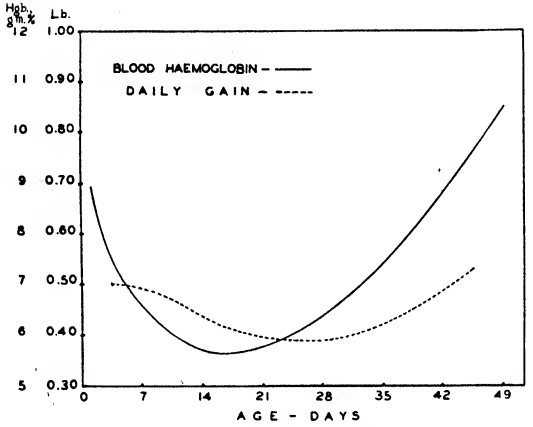


FIG. 1. *Blood haemoglobin and daily gain of 61 pigs born in January (Expt. I).*

The trend of the blood haemoglobin followed the expected curve (according to other workers, including Kernkamp (5), Moe, Craft, and Thompson (6)). The downward trend of the daily gain to the fourth week of life indicated a possible relationship of lower haemoglobin levels to growth.

Results

Tests were conducted with the next lot of litters that were born during May and June, 1941, in order to determine the effects of keeping the blood haemoglobin at higher concentrations. Two forms of iron were used for comparison of values; ferrous sulphate ($FeSO_4 . 2H_2O$) and iron reduced by hydrogen were chosen. The dose of ferrous sulphate was set at $4\frac{1}{2}$ grains (= 86 mg. iron) and that of reduced iron was set at 107 mg. Eight litters, comprising 75 pigs, were used, each litter being divided at random into three lots. One three grain and one one and one-half grain coated tablet of ferrous sulphate was given as the dose to each of one group, one capsule containing 107 mg. of reduced iron to each of the second group, and the third group was not dosed.** Bleeding for haemoglobin readings, administration of iron preparations and recording of body weights were first made on each litter between the 12th and 36th hour after birth and at weekly intervals thereafter to seven weeks.

* *Ashton, G. C. and Crampton, E. W. Rates of growth of bacon-type nursing pig. (Unpublished manuscript.)*

** *The iron preparations used for this experiment were supplied by the Veterinary Department of Ayerst, McKenna, and Harrison Ltd., Montreal.*

of age. The weekly data obtained were subjected to an analysis of variance according to the scheme shown on Table II.

TABLE II

ANALYSIS OF VARIANCE OF LIVE WEIGHTS OF TWO-DAY-OLD PIGS

Variance due to:	D.f.	Sums of squared deviations $S(x - \bar{x})^2$	Variance
All causes	74	21.68	
Between sows	7	5.35	0.76
Between treated groups	2	0.44	0.22
Between sexes	1	0.54	0.54
Remainder	64	15.36	0.24

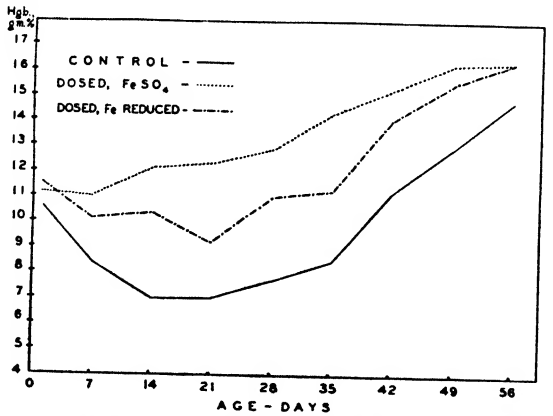


FIG. 2. *Blood haemoglobin of 75 pigs born in May and June (Expt. II).*

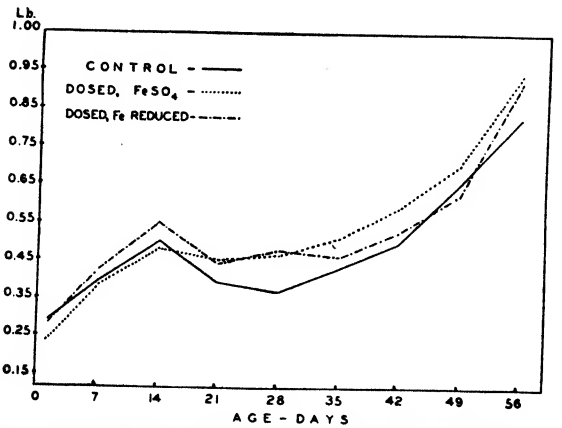


FIG. 3. *Daily gain of 75 pigs born in May and June (Expt. II).*

The mean weekly live weights and haemoglobin levels are shown in Table III, the latter also appearing as plotted curves in Fig. 2. The daily gains (as observed) are shown in Fig. 3.

TABLE III

MEAN WEEKLY LIVE WEIGHTS AND HAEMOGLOBIN LEVELS OF 75 PIGS BORN IN MAY AND JUNE, 1941, AND SUBJECTED TO IRON THERAPY

Treatment	Observations	Age (days)								
		1	7	14	21	28	35	42	49	56
Check	Haemoglobin, gm.%	10.7	8.4	7.0	7.0	7.8	8.5	11.2	13.0	14.9
	Weight (lb.)	3.2	5.4	8.8	11.6	15.2	17.2	20.7	25.3	30.7
Ferrous sulphate	Haemoglobin	11.2	11.0	12.1	12.3	12.9	14.3	15.3	16.3	16.4
	Weight	3.0	5.2	8.6	11.9	16.5	18.6	22.6	27.6	33.6
Reduced iron	Haemoglobin	11.6	10.1	10.4	9.2	11.0	11.3	14.1	15.6	16.4
	Weight	3.2	5.5	9.5	12.5	17.4	19.0	22.7	27.3	33.1

In estimating the results in terms of statistical analysis, Table IV was prepared because of the lack of definite differences at all stages of growth. Because the differences between groups were not statistically significant (at a probability (P) = 0.05) at all periods, the odds that the observed differences were due to treatment were calculated. In Table IV it will be noted that at 43, 50, and 56 days, the odds are 20 to 1 or better that the differences between the untreated and treated groups are real.

TABLE IV

EXPT. II. DIFFERENCES IN LIVE WEIGHTS OF PIGS AT SPECIFIED AGES

Age (days)	Check group vs. $FeSO_4$ group			Check group vs. Fe reduced group			$FeSO_4$ group vs. Fe reduced group		
	Differences		Observed P	Differences		Observed P	Differences		Observed P
	Obs.	Necessary (P=0.05)		Obs.	Necessary (P=0.05)		Obs.	Necessary (P=0.05)	
2	0.17	0.27	0.20	0.00	—	—	0.16	0.28	0.25
7	0.19	0.51	0.45	0.15	0.52	0.55	0.34	0.52	0.20
15	0.23	0.80	0.57	0.70	0.81	0.10	0.90	0.82	0.04
22	0.23	1.11	0.68	0.90	1.12	0.10	0.60	1.13	0.21
29	1.30	1.38	0.07	2.20	1.40	0.01	0.90	1.41	0.18
36	1.37	1.62	0.09	1.80	1.64	0.03	0.40	1.66	0.60
43	1.95	1.86	0.03	1.98	1.87	0.03	0.02	1.90	0.90
50	2.33	2.40	0.05	2.02	2.42	0.10	0.31	2.45	0.80
56	2.87	2.76	0.04	2.39	2.73	0.08	0.48	2.76	0.73

It is apparent that both forms of iron were effective in keeping the haemoglobin levels well above those of the undosed group, ferrous sulphate having shown a slight superiority in this respect. Both groups of dosed pigs appeared, to the casual observer, to be somewhat more thrifty and of a better colour than the control group. The difference between the average weaning weight of the group receiving ferrous sulphate and the control group was significant, as we have seen. The difference between the group receiving reduced iron and the control group closely approached the point of "significance"; there was no statistically significant difference between the treated groups (ferrous sulphate vs. reduced iron).

Discussion

The observations on both lots of pigs added support to the data of previous years that show a period of slower growth in suckling pigs between the first and fourth weeks of life. This flattening of the growth curve was modified by iron therapy in the experiment and thus it seems probable that a lowered concentration of blood haemoglobin, not reaching the point of clinical anaemia, has a direct effect upon gain in weight.

The possibility that cyclic periods of growth may be normal in pigs must not be overlooked, particularly in view of the data for other young animals as presented by Brody *et al.* (1). However, if such a phenomenon occurs, then the cyclic decrease in rate of gain in suckling pigs can be influenced by a dietary supplement that keeps the blood haemoglobin at a higher concentration.

The practical importance of the results lies in the finding of the significant increase in body weight at weaning time in the group that received the weekly doses of an iron salt. It is apparent that the thriftiness of the control group was adversely affected as a result of the drop in haemoglobin, even though no clinical symptom of anaemia appeared and no haemoglobin level suggesting clinical anaemia was found. If normal growth of suckling pigs is affected, then it is probable that resistance to infection by animal parasites and other organisms would be lowered.

The drop in haemoglobin during the first week of life is considerable and as this work has indicated the benefits of preventing this initial fall, measures of health protection should include the administration of some form of iron to pigs during the first day or two after birth. There is no mechanical difficulty in administering the tablets or capsules, as a new-born pig readily swallows even a No. 00 gelatine capsule with a sucking motion if it is placed on the tongue and the finger retained in the mouth for a few seconds.

It is now generally recognized that a pig is able to utilize various iron preparations to a wider extent than humans. The two forms generally used are the ones employed in the experiment; ferrous sulphate is low in cost and has a theoretical advantage in having traces of copper as an impurity, which acts as a catalyst in the utilization of iron. However, the commercial undried ferrous sulphate (copperas) contains only slightly more than 20% iron, whereas reduced iron contains 90 to 96% metallic iron; thus the greater bulk

is a factor for consideration. The many factors that influence the utilization of iron cannot be discussed here, but the dosages used are apparently safe and effective for the purpose. Overdosage must be avoided, as toxic effects are possible sequelae. For practical application it is probable that the doses of either preparation could safely be reduced in number to two, one soon after birth and one about 10 days later, as advised by Schofield.

Pigs may eat a small amount of soil very soon after birth, thus the practical method of providing soil fortified with an iron salt is not to be discouraged as a means of attaining the desired results.

Work on this and other possible factors in resistance to disease in suckling pigs is being continued.

References

1. BRODY, S. Missouri Agr. Expt. Sta. Research Bull. 62. 1923.
2. CHOQUETTE, L. Unpublished data. 1940–42.
3. FISHER, R. A. Statistical methods for research workers. 6th ed. rev. and enl. Oliver and Boyd, Edinburgh and London. 1936.
4. HAMILTON, T. S., HUNT, G. E., and CARROLL, W. E. J. Agr. Research, 47(8) : 543-563. 1933.
5. KERNKAMP, H. C. H. J. Am. Vet. Med. Assoc. 87(1) : 37-58. 1935.
6. MOE, L. H., CRAFT, W. A., and THOMPSON, C. P. J. Am. Vet. Med. Assoc. 87(3) : 302-311. 1935.
7. SCHOFIELD, F. W. Rept. Ontario Vet. Coll., 1929, pp. 44-50. 1929.
8. SCHOFIELD, F. W. Rept. Ontario Vet. Coll., 1936, pp. 16-17. 1936.
9. WHITBY, L. E. H. and BRITTON, C. J. C. Disorders of the blood. 3rd ed. J. & A. Churchill, Ltd., London. 1939.

SECTION D

INDEX TO VOLUME 20

Authors

Allen, D. E.—See Collier, H. B. and Swales, W. E.

Armstrong, F. H.—See Hayes, F. R.

Ashton, G. C.—See Swales, W. E.

Battle, H. I.—Hepatogenesis of the Atlantic salmon (*Salmo salar* L.), 79.

Berkeley, C.—See Berkeley, E.

Berkeley, E. and Berkeley, C.—North Pacific Polychaeta, chiefly from the west coast of Vancouver Island, Alaska, and Bering Sea, 183.

Black, V. S.—The effect of asphyxiation under various tensions of carbon dioxide on the swim bladder gases of some freshwater fish, 209.

Buckby, L.—See Reedman, E. J.

Charnley, F. and Goard, D. H.—On the use of the pH value as a measure of the freshness of fish muscle tissue, 20.

Choquette, L.—See Swales, W. E.

Collier, H. B.—See Swales, W. E.

Collier, H. B. and Allen, D. E.—The haemolytic action of phenothiazine derivatives, 283.

Cook, W. H.—See White, W. H.

Crampton, E. W.—See Swales, W. E.

Crandall, W. A.—See Pugsley, L. I.

Dunbar, M. J.—Marine macroplankton from the Canadian eastern Arctic.
I. Amphipoda and Schizopoda, 33.
II. Medusae, Siphonophora, Ctenophora, Pteropoda, and Chaetognatha, 71.

Fallis, A. M.—The life cycle of *Apanteles carpatus* (Say) (Hymenoptera:Braconidae), a parasite of the webbing clothes moth, *Tineola bisselliella* Hum., 13.

Fisher, K. C.—The effect of temperature on the critical oxygen pressure for heart-beat frequency in embryos of Atlantic salmon and speckled trout, 1.

Fletcher, D. A.—See Rosser, F. T.

Fulton, C. O.—See Gibbons, N. E.

Gibbons, N. E.—See White, W. H.

Gibbons, N. E., Fulton, C. O., and Hopkins, J. W.—Preservation of eggs. IV. Storage of oiled and bagged eggs under constant and alternating temperatures and humidities, 306.

Glenny, F. H.—Main arteries in the region of the neck and thorax of the Australian cassowary, 363.

Goard, D. H.—See Charnley, F.

Gwatkin, R. and Moynihan, I. W.—Search for sources and carriers of equine encephalomyelitis virus, 321.

Hayes, F. R. and Armstrong, F. H.—Physical changes in the constituent parts of developing salmon eggs, 99.

Hopkins, J. W.—See Gibbons, N. E. and Reedman, E. J.

Kelly, J. T.—See Pugsley, L. I.

Legault, R. T.—See Reedman, E. J.

Little, G. E.—See McLeod, J. A.

McLeod, J. A. and Little, G. E.—Continued studies on cercarial dermatitis and the trematode family Schistosomatidae in Manitoba. Part I, 170.

Maw, W. A. and Nikolaiczuk, N.—The effect of ethyl alcohol and tricresol on the vitamin A content of the blood and liver of the chicken, 47.

Morrell, C. A.—See Pugsley, L. I.

Moynihan, I. W.—See Gwatkin, R.

Munro, J. A.—Studies of waterfowl in British Columbia. Buffle-head, 133.

Nikolaiczuk, N.—See Maw, W. A.

Pearce, J. A. and Thistle, M. W.—Fluorescence as a measurement of quality in dried whole egg powder, 276.

Peters, H. S.—See Shortt, T. M.

Pugsley, L. I., Kelly, J. T., Crandall, W. A., and Morrell, C. A.—The vitamins A and D potency of the oils obtained from the liver, intestines, body, and offal of shad, *Alosa sapidissima* Wilson, and mackerel, *Scomber scombrus* L., 167.

Reed, G. B. and Toner, G. C.—*Proteus hydrophilus* infections of pike, trout, and frogs, 161.

Reedman, E. J., Buckby, L., and Legault, R. T.—The vitamin C (ascorbic acid) content of canned chicken, 230.

Reedman, E. J. and Hopkins, J. W.—Preservation of eggs. III. Studies on the effect of oil treatment and egg-case liner bags in the preservation of shell eggs under adverse conditions, 297.

Rosser, F. T.—Preservation of eggs. II. Surface contamination on egg-shell in relation to spoilage, 291.

Rosser, F. T., White, W. H., Woodcock, A. H., and Fletcher, D. A.—Preservation of eggs. I. Treatments for maintaining quality in shell eggs at ordinary temperatures, 57.

Russell, H. D.—A new species of *Onchidiopsis* from Baffin Land, 50.

Shortt, T. M. and Peters, H. S.—Some recent bird records from Canada's eastern Arctic, 338.

Smith, S. G.—Polarization and progression in pairing.

II. Premeiotic orientation and the initiation of pairing, 221.

III. Pachytene observations in *Neodiprion* (Hymenoptera), 368.

Swales, W. E., Collier, H. B., and Allen, D.—Effects of phenothiazine on horses. I. Studies in haematology and pharmacology, 349.

Swales, W. E., Crampton, E. W., Ashton, G. C., and Choquette, L.—Studies on factors influencing the health of pigs. I. The relationship of blood haemoglobin concentrations to rate of gain in suckling pigs, 380.

Swales, W. E., Sylvestre, P. E., and Williams, S. B.—Field trials of control measures for parasitic disease of sheep, 115.

Sylvestre, P. E.—See Swales, W. E.

Thistle, M. W.—See Pearce, J. A.

Toner, G. C.—See Reed, G. B.

Walker, M. G.—A mathematical analysis of the distribution in maize of *Heliothis armigera* Hb. (*obsoleta* F.), 235.

White, W. H.—See Rosser, F. T.

White, W. H., Gibbons, N. E., Woodcock, A. H., and Cook, W. H.—Smoked meats. I. Bacteriological, chemical, and physical measurements on smoked and unsmoked bacon, 263.

Williams, S. B.—See Swales, W. E.

Woodcock, A. H.—See Rosser, F. T. and White, W. H.

SECTION D

INDEX TO VOLUME 20

Subjects

Alcohol, Ethyl, Effect on vitamin A content of blood and liver of chicken, 47.

Alosa sapidissima, See Shad.

Ameiurus nebulosus, See Bullhead.

Amnicola, examined for presence of cercariae, 174.

Amphipoda, See Plankton.

Anaemia
produced in horses by phenothiazine, 349.
Rate of gain of young pigs in absence of, 380.

Anthelmintic(s)
as control measures for parasitic diseases of sheep, 115.
Effect of phenothiazine on horses when used as, 349.

Apanteles carpatus, See Insects.

Arctic, Canada's eastern, Bird records from, 338.

Arteries in region of neck and thorax of Australian cassowary, 363.

Ascorbic acid content of canned chicken, 230.

Asphyxiation, Effect of, on swim bladder gases of freshwater fish, 209.

Assassin bug, See Insects.

Bacon, Smoked and unsmoked, Bacteriological, chemical, and physical measurements on, 263.

Bacteria
As egg contaminants, 293, 300, 307.
Effect of concentration of sodium chloride in cover pickle on growth of, 267.
Effect of method of cure and other factors on growth of, on bacon, 269.
Proteus hydrophilus infections of fish and frogs, 161.

Bass, Northern smallmouth, Effect of asphyxiation on swim bladder gases of, 209.

Bile salt haemolysis of horse erythrocytes, 288.

Birds
Arteries in region of neck and thorax of Australian cassowary, 363.
Records of, from Canada's eastern Arctic, 338.
See Buffle-head.

Blood
Chicken, Effect of ethyl alcohol and tricresol on vitamin A content of, 47.
haemoglobin concentrations, Relationship of, to rate of gain in suckling pigs, 380.
Horse, Effect of phenothiazine on, 283, 349.
Sheep, Effect of phenothiazine on, 283.
vessels, See Arteries.

Body of shad and mackerel, Vitamin A and D potency of, 167.

Boll-worm, See Insects.

Braconidae, See Insects (Apanteles).

British Columbia, Studies of waterfowl in, 133.

Buffle-head in British Columbia
Behaviour of females and young, 146.
Colour of soft parts of, 151.
Courtship of, 140.
Food and feeding habits, 152.
Mating of, 142.
Moults and plumages of, 148.
Sex ratio of, 147.
Spring and autumn migration of, 134, 138.
Summer and winter populations of, 134, 138.

Bug, See Insects.

Bullhead, Northern brown, Effect of asphyxiation on swim bladder gases of, 209.

Canada, Bird records from eastern Arctic region of, 338.

Carbon dioxide
Effect of asphyxiation under various tensions of, on swim bladder gases of freshwater fish, 209.
Use of, in preserving eggs, 57.

Carotene in blood and liver of chicken' Effect of ethyl alcohol and tricresol on' 47.

Cassowary, Australian, Main arteries in region of neck and thorax of, 363.

Casuarius australis, Arteries of, 363.

Cat, as possible carrier of encephalomyelitis, 321.

Catostomus commersonii, See Sucker.

Cells, See Erythrocytes.

Cercaria, Species of, in snails and other hosts, 174, 175, 182.

Cercaria dermolestes, Relation to life cycle of Pseudobilharziella, 180.

Cercarial dermatitis, See Dermatitis.

Cercaria physella, Relation of Pseudobilharziella querquedulae to, 180.

Chaetognatha from the Canadian eastern Arctic, 75.

Charitonetta albeola in British Columbia, 133.

Chicken
- Blood and liver of, Effect of ethyl alcohol and tricresol on vitamin A content of, 47.
- Canned, Vitamin C content of, 230.
- Non-infection of, by cercariae, 175.

Chromosomes
- of Diptera, Premeiotic orientation and initiation of pairing in, 221.
- of Neodiprion, Observations of, at pachytene, 368.

Chub, Northern creek, Effect of asphyxiation on swim bladder gases of, 209.

Citellus richardsoni, See Squirrel.

Colour of bacon, Changes in, during storage, 273, 274.

Cooperia, Control of, in sheep, 115.

Corn, Analysis of distribution of Heliothis armigera in, 235.

Crickets, See Insects.

Crow, as possible carrier of encephalomyelitis, 321.

Ctenophora from the Canadian eastern Arctic, 75.

Culex tarsalis, See Insects (Mosquito).

Cythaemolytic icterus produced in horses by phenothiazine, 349.

Dace, Finescale, Effect of asphyxiation on swim bladder gases of, 209.

Dafila acuta, Pseudobilharziella in, 180.

Dermacentor andersoni, See Insects (Tick).

Dermatitis, Cercarial, and the trematode family Schistosomatidae in Manitoba, 170.

Dimethylol urea, Use of, in preserving eggs, 57.

Diptera, See Insects.

Duck
- As possible carrier of encephalomyelitis, 321.
- Pin-tail, Occurrence of Pseudobilharziella in, 180.
- Sea, See Buffle-head.
- Spoon-billed, Occurrence of Pseudobilharziella in, 180.
- Teal, Non-infection of, by cercariae, 175.

Egg(s)
- Oiled and bagged, Storage of, under constant and alternating temperatures and humidities, 306.
- powder, Fluorescence as a measurement of quality in, 276.
- Preservation of, as affected by oil treatment and egg-case liner bags under adverse conditions, 297.
- Salmon
  - Changes in capsule of, during development, 101.
  - Physical changes in developing, 99.
- Shell of, Surface contamination of, in relation to spoilage, 291.
- Sterile artificial, Method of preparing, 59.
- Treatment of, for storage experiments, 57, 292, 298, 307.

Embryo
- of salmon, Changes in, during development, 109.
- of salmon and trout, Effect of temperature on critical oxygen pressure for heartbeat frequency in, 1.

Encephalomyelitis virus, Search for sources and carriers of, 321.

Erythrocytes
- of horse blood, Effect of phenothiazine on, 283, 349.
- of sheep blood, Effect of phenothiazine on, 283.

Eucalia inconstans, See Stickleback.

Ferrous sulphate, Effect of, on weight of young pigs, 380.

Fish
Freshwater, Effect of asphyxiation on swim bladder gases of, 209.
Mackerel oil, Vitamin A and D potency of, 167.
Muscle tissue of, Use of pH as measure of, 20.
Salmon
Development of liver in, 79.
eggs, Physical changes in developing, 99.
embryos, Effect of temperature on critical oxygen pressure for heart-beat frequency in, 1.
Trout, Proteus hydrophilus infections of, 161.

Flavour
of bacon, Effect of smoking on, 273.
of dried egg powder, Fluorescence as a measure of, 279.

Fluorescence as a measurement of quality in dried whole egg powder, 276.

Frogs, Proteus hydrophilus infections of, 161.

Fungi
as egg contaminants, 293.
Inhibition of growth of, on eggs at ordinary temperatures, 57.

Gases, Swim bladder, of freshwater fish, Effect of asphyxiation on, 209.

Gastropod, A new species of, from Baffin Land, 50.

Grasshoppers, See Insects.

Ground squirrel, as possible carrier of encephalomyelitis, 321.

Gulls, Herring, Non-infection of, by cercariae, 175.

Gyraulus parvus, examined for species of cercariae, 174.

Haemoglobin, See Blood.

Haemolysis, Effect of phenothiazine on, in horses, 283, 349.

Haemonchus, Control of, in sheep, 115.

Hawk, as possible carrier of encephalomyelitis, 321.

Heart-beat frequency in embryos of salmon and trout, Effect of temperature on the critical oxygen pressure for, 1.

Heliosoma trivolvis, examined for presence of cercariae, 174.

Heliothis, See Insects (Boll-worm).

Helminth parasites, See Parasites.

Hen, as possible carrier of encephalomyelitis, 321.

Hepatogenesis of the Atlantic salmon, 79.

Horse, See Blood and Virus.

Humidity, Effect of, on preservation of eggs, 306.

Hybocodon prolifer, a new record from Arctic water, 71.

Hymenoptera, See Insects.

Insects
Apanteles carpatus (Hymenoptera), Life cycle of, 13.
Assassin bug, as possible carrier of encephalomyelitis, 321.
Boll-worm, Analysis of distribution of, in maize, 235.
Crickets, as possible carriers of encephalomyelitis, 321.
Diptera, Premeiotic orientation and initiation in pairing in chromosomes of the, 221.
Grasshoppers, as possible carriers of encephalomyelitis, 321.
Mosquito, as possible carrier of encephalomyelitis, 321.
Moth, Webbing clothes, Life cycle of Apanteles, a parasite of, 13.
Neodiprion, Pachytene observations at meiosis in, 368.
Stomoxys, as possible carrier of encephalomyelitis, 321.
Tabanidae, Members of, as possible carriers of encephalomyelitis, 321.
Ticks, as possible carriers of encephalomyelitis, 321.

Intestines of shad and mackerel, Vitamin A and D potency of, 167.

Iron, Reduced, Effect of, on weight of young pigs, 380.

Larva, Salmon, Changes in, during development, 108.

Lepidoptera, See Insects.

Lepomis gibbosus, See Pumpkin-seed.

Light, Effect of, on haemolysis of horse erythrocytes in presence of phenothiazine derivatives, 288.

Liver
of chicken, Effect of ethyl alcohol and tricresol on vitamin A content of, 47.
of mackerel, Vitamin A and D potency of, 167.
of salmon
Development of, 79.
Morphology of, 94.
of shad, Vitamin A and D potency of, 167.

Lymnaea stagnalis jugularis, examined for presence of cercariae, 174.

Lysolecithin, Haemolysis of erythrocytes by, 287.

Mackerel, Vitamin A and D potency of oil of, 167.

Macroplankton, See Plankton.

Maize, Mathematical analysis of distribution of Heliothis armigera in, 235.

Meat, See Bacon and Chicken.

Medusae from the Canadian eastern Arctic, 71.

Meiosis
Pachytene observations in Neodiprion, 368.
Premeiotic orientation and the initiation of pairing in Diptera, 221.

Metopa longirama, a new amphipod species from the Canadian eastern Arctic, 39.

Micro-organisms, See Bacteria and Fungi.

Micropterus dolomieu, See Bass.

Mollusc(s)
A new, from Baffin Land, 50.
from the Canadian Arctic, 75.

Moniezia, Control of, in sheep, 115.

Monodontus, Control of, in sheep, 115.

Mosquito, See Insects.

Moth, See Insects.

Moulds
as egg contaminants, 293, 300, 307.
Inhibition of growth of, on eggs at ordinary temperature, 57.

Mus, See Rat.

Muscle tissue of fish, Value of pH as measure of, 20.

Nematodirus, Control of, in sheep, 115.

Neodiprion, See Insects.

Oesophagostomum, Control of, in sheep, 115.

Offal of shad and mackerel, Vitamin A and D potency of, 167.

Oils of shad and mackerel, Vitamins A and D potency of, 167.

Onchidiopsis kingmaruensis, New species of mollusc from Baffin Land, 50.

Oncorhynchus Keta, Use of pH as measure of freshness in, 24.

Ostertagia, Control of, in sheep, 115.

Owl, as possible carrier of encephalomyelitis, 321.

Oxygen
in swim bladder of fish after asphyxiation, 209.
pressure, Effect of temperature on, for heart-beat frequency in embryos of salmon and trout, 1.

Parasites
Helminth, Control of, in sheep, 115.
Life cycle of Apanteles carpatus, parasite of webbing clothes moth, 13.

Penicillium citrinum, Inhibition of growth of, on eggs, 60.

Perca flavescens, See Perch.

Perch, Yellow, Effect of asphyxiation on swim bladder gases of, 209.

Pfrille neogaea, See Dace.

pH, Use of, as measure of freshness of fish muscle tissue, 20.

Pheasant, as possible carrier of encephalomyelitis, 321.

Phenothiazine
Effect of, on horses, 349.
Effect of derivatives of, on haemolysis, 283.

Photofluorometer, Measurement of fluorescence of egg powder by, 277.

Physella gyrina, examined for presence of cercariae, 174.

Physoclists, Effect of asphyxiation on swim bladder gases of, 209.

Physostomes, Effect of asphyxiation on swim bladder gases of, 209.

Pigeons, Non-infection of, by cercariae, 175.

Pigs
as possible carriers of encephalomyelitis, 321.
Relationship of blood haemoglobin concentrations to rate of gain in suckling, 380.

Pike, Proteus hydrophilus infections of, 161.

Pisobia malanotos, examined for presence of cercariae, 175.

Pista
moorei, new polychaete species from North Pacific, 204.
pacifica, new polychaete species from North Pacific, 202.

Plankton, Marine macroplankton from the Canadian eastern Arctic.
I. Amphipoda and Schizopoda, 33.
II. Medusae, Siphonophora, Ctenophora, Pteropoda, and Chaetognatha, 71.

Polarization and progression in pairing
in Diptera, 221.
in Neodiprion, 368.

***Polychaeta** from the North Pacific, 183.*

Polyvinyl alcohol, Use of, in preserving eggs, 57.

Pressure, Critical oxygen, See Oxygen.

Proteus hydrophilus
Biochemical reactions of culture of, 164, 165.
Comparison of, from various sources, 164, 166.
Infections of pike, trout, and frogs by, 161.

Pseudobilharziella querquedulae, Completion of life cycle of, 170.

Pseudosuccinea sp., examined for presence of cercariae, 174.

Pteropoda from the Canadian eastern Arctic, 75.

Pumpkin-seed, Effect of asphyxiation on swim bladder gases of, 209.

Rabbit, as possible carrier of encephalomyelitis, 321.

Rancidity, Development of, in bacon during storage, 271.

Rat, as possible carrier of encephalomyelitis, 321.

Red leg of frogs, Proteus hydrophilus as cause of, 162.

Red sore of pike, Proteus hydrophilus as cause of, 161.

Salmon
Atlantic, Hepatogenesis of, 79.
Chum, Use of pH as measure of freshness in, 24.
eggs, Physical changes in developing, 99.
embryos, Effect of temperature on critical oxygen pressure for heart-beat frequency in, 1.

Salmo salar, See Salmon.

Salvelinus fontinalis, See Trout.

Sandpiper, Pectoral, examined for presence of cercariae, 175.

Saponin, Haemolysis of erythrocytes by, 285, 286.

Schistosomatidae and **cercarial dermatitis** in Manitoba, 170.

Schizopoda, See Plankton.

Scionella estevanica, new polychaete species from the North Pacific, 205.

Scomber scombrus, See Mackerel.

Sea duck, See Buffle-head.

***Semotilus atromaculatus,** See Chub.*

Shad, Vitamin A and D potency of oil of, 167.

Sheep
Control measures for parasitic diseases of, 115.
See Blood.

Sinea diadema, See Insects (Assassin bug).

Siphonophora from the Canadian eastern Arctic, 75.

Snails, Species of, examined for presence of cercariae, 174.

Spatula clypeata, Pseudobilharziella in, 180.

Spoilage of eggs
Effect of oil treatment and egg case liner bags on, during storage, 297.
stored under constant and alternating temperatures and humidity, 310.
Surface contamination on egg-shell in relation to, 291.

Spot rot found in eggs, 300.

Stagnicola, Species of, examined for presence of cercariae, 174.

Stickleback, Brook, Effect of asphyxiation on swim bladder gases of, 209.

Stomoxys, See Insects.

Storage of eggs, See Spoilage.

Sucker, Common white, Effect of asphyxiation on swim bladder gases of, 209.

Swim bladder of freshwater fish, Effect of asphyxiation on gases of, 209.

Syllis stewarti, new polychaete species from the North Pacific, 191.

Tabanidae, Members of, as possible carriers of encephalomyelitis, 321.

Temperature(s)
Effect of, on critical oxygen pressure for heart-beat frequency in embryos of salmon and trout, 1.
Optimum, for Proteus hydrophilus, 163.
Ordinary, Treatment to maintain quality in eggs at, 57.
Preservation of eggs at varying, 306.

Ticks, See Insects.

Tineola bisselliella, Life cycle of Apanteles carpatus, a parasite of, 13.

Totanus flavipes, examined for presence of cercariae, 175.

Trematode(s) and cercarial dermatitis in Manitoba, 170.

Trichostrongylus, Control of, in sheep, 115.

Tricresol, Effect of, on vitamin A content of blood and liver of chicken, 47.

Trout
- Common brook, Effect of asphyxiation on swim bladder gases of, 209.
- Embryos, Effect of temperature on critical oxygen pressure for heart-beat frequency in, 1.
- Proteus hydrophilus infections of, 161.

Ulcer disease of trout, Proteus hydrophilus, as cause of, 161.

Valvata tricarinata, examined for presence of cercariae, 174.

Vaseline, Use of, in preserving eggs, 57.

Virus, Search for sources and carriers for equine encephalomyelitis, 321.

Vitamins
- Vitamin A
  - in blood and liver of chicken, Effect of ethyl alcohol and tricresol on, 47.
  - potency of shad and mackerel oil, 167.
- Vitamin C content of canned chicken, 230.
- Vitamin D potency of shad and mackerel oil, 167.

Water, Lake, Proteus hydrophilus in, 162.

Waterfowl, Studies of, in British Columbia, 133.

Water hardening of salmon eggs, 102.

Worms, See Parasites.

Yellow-legs, Lesser, examined for presence of cercariae, 175.

Yolk sac of salmon egg, 109.

Zelus audax, see Insects (Assassin bug).

CANADIAN JOURNAL OF RESEARCH

VOLUME 21
1943
SECTION D

CANADA

Published by the
NATIONAL
RESEARCH COUNCIL
of CANADA

19228

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. JANUARY, 1943 NUMBER 1

DRIED WHOLE EGG POWDER

I. METHODS OF ASSESSING QUALITY[1]

BY M. W. THISTLE[2], J. A. PEARCE[2], AND N. E. GIBBONS[3]

Abstract

Several methods of assessing quality in dried whole egg powders were studied on a wide range of material from Canadian egg drying plants. Moisture content and bacterial count varied independently, and are regarded as necessary measures of quality. Beating value, pH of egg batter, water value, potassium chloride value, fluorescence measurements, and palatability ratings were all significantly interrelated. Of these methods, potassium chloride value and fluorescence measurements were the most sensitive and also the most closely associated with palatability ratings.

Introduction

The necessity of transporting eggs to Britain in the dried form, in order to supply a less perishable product than shell eggs and also to save shipping space, has resulted in an investigation of methods of estimating and controlling the quality of the first-class dried whole egg powders required for domestic consumption. This paper describes and assesses the methods of measuring quality that appeared to be useful when the work was undertaken. Methods other than those described are under test.

Materials

The relative suitability of the various methods for assessing quality could best be studied on samples showing a wide range of quality. However, it was also desirable to meet the conditions of a practical test. To fulfil these requirements, two sets of samples were obtained, prime quality powders secured weekly from five Canadian plants for a period of six weeks, and a range of inferior material consisting of 19 residue samples, obtained from the secondary dust collectors of four of the plants.

Methods

All methods of assessing quality in egg powder that appeared to be useful when this study was undertaken were used concurrently on the 49 samples. Details of these tests follow.

[1] *Manuscript received October 17, 1942.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as paper No. 84 of the Canadian Committee on Food Preservation and as N.R.C. No. 1097.

[2] *Biochemist, Food Investigations.*

[3] *Bacteriologist, Food Investigations.*

19228

Moisture as Total Volatiles

The method used was a modification of a standard A.O.A.C. procedure (1, p. 308): duplicate 10-gm. samples were heated in a vacuum oven at 100° C. for 12–16 hr. or overnight. These conditions suited laboratory practice, and it was found that the extra time did not materially affect the results.

Bacterial Count

Five grams of powder was ground in a mortar with 10 gm. of sand and 45 ml. of water to give a homogeneous emulsion. After dilution, aliquots were plated in duplicate on a medium composed of 0.5% proteose–peptone (Difco); 0.5% tryptone (Difco), and 1.5% agar. The pH was adjusted to 6.8–7.0 if necessary. Counts were made after three days' incubation at 37° C.

Counts were also made after seven days' incubation at 20° C., but as they were similar to three-day counts at 37° C., the longer incubation period was discontinued.

Beating Value

The method was a modification of that in use at the Low Temperature Research Station, Cambridge, England*. Eighteen grams of egg powder was mixed with 60 gm. of sugar, placed in a small "Mixmaster" bowl, and reconstituted by adding 57 ml. of distilled water and mixing at the lowest speed of the mixer for five minutes, followed by 20 min. mixing at the highest speed. The volume of the resulting material constituted the beating value.

pH on Residue from Beating Test

Rather than make separate solutions for pH measurements, it appeared advisable to use left-over solutions from other tests. Materials from the water value, potassium chloride value, and beating tests have all been used, but in this particular series use was made of the residue from the beating test.

Measurements were made with the Beckman pH meter, using a glass electrode and a saturated calomel half-cell.

Water Value

It may be interjected at this point that water value and potassium chloride value (to be discussed) take into account possible protein–lipoid associations, and therefore should not be confused with those solubility tests that are concerned with protein only.

An accurately weighed portion of about 2.2 gm. of powder was shaken for an hour in 100 ml. of distilled water, and filtered through a No. 1 Whatman filter paper. An aliquot of 20 ml. of filtrate was transferred to a weighed beaker and coagulated on a hot-plate. The coagulated material was then dried at 110° C. for 16 hr., and the residue weighed and expressed as a percentage of the original weight of sample.

* *Private communication.*

Potassium Chloride Value

The procedure was the same as that described for the water value, except that 10% potassium chloride solution replaced water. The weight of dissolved salt was subtracted, and the residual weight expressed as a percentage of the weight of sample taken.

Fluorescence Measurements

Briefly, 2.5 gm. of defatted egg powder (fat extracted with chloroform) was shaken with 10% potassium chloride, and the fluorescence of the extract measured with a Coleman photofluorometer. Details of the method have been described (2).

Palatability Test

Details have also been given for this test (2). Each sample, mixed with water and coagulated in a water-bath, was tasted by a panel of 13 people. The original British system of scoring was followed, ranging from 10 for excellent, fresh egg to 0 for repulsive material. Recently more elaborate work on the palatability test has been published (3).

Results

Mean values for all measurements on material from different plants are given in Table I. Fluorescence measurements were more precise, and distinguished more sharply between powders from different plants than the other quality tests. Potassium chloride value is also indicated as being among the more sensitive tests.

TABLE I

MEAN VALUES OF QUALITY MEASUREMENTS ON EGG POWDERS FROM DIFFERENT PLANTS

Groups tested	Plant No.	Methods							
		Volatile materials, %	Bacterial count, log	Beating value, ml.	pH	Water value, %	KCl value, %	Fluorescence, units	Palatability, score
Prime quality samples	1	4.41	4.75	237	8.36	76.9	70.7	19.2	7.35
	2	4.35	5.80	260	8.43	75.9	75.5	15.9	8.03
	3	3.27	5.35	235	8.44	74.7	76.9	19.2	7.58
	4	5.70	6.27	251	8.31	73.4	75.5	18.8	7.71
	5	4.39	4.01	258	8.45	71.8	81.1	19.9	7.56
Necessary difference[1]		0.05	0.69	*n.s.d.*[2]	*n.s.d.*[2]	1.8	1.6	0.2	*n.s.d.*[2]
Dust-collector samples	1	3.08	4.67	249	8.23	71.1	63.1	30.0	6.11
	2	4.12	5.15	173	7.94	54.4	38.4	46.8	5.17
	3	3.49	5.57	160	7.90	51.2	33.7	50.9	5.20
	4	5.78	6.24	250	8.25	73.9	69.0	21.0	7.18
Necessary difference[1]		0.17	0.72	39	0.23	4.8	3.4	1.7	0.63

[1] *Necessary difference at the 5% level of significance.*

[2] *No significant difference between plant averages on prime quality samples.*

The significance of the over-all quality differences between plants and between sampling times was assessed by means of analyses of variance, given in Tables II and III. The objective tests, shown in Table II, generally distinguished average differences between plants on prime samples, whereas palatability ratings did not, as shown in Table III. On dust-collector materials, on the other hand, all tests showed marked differences between plant averages.

TABLE II

ANALYSES OF VARIANCE OF QUALITY IN EGG POWDERS, AS ASSESSED BY OBJECTIVE TESTS

Groups tested	Variance attributable to:	Degrees of freedom	Mean square						
			Volatile materials	Bacterial count	Beating value	pH	Water value	Potassium chloride value	Fluorescence measurements
Prime quality samples	Plants	4	8.90**	4.70**	818	0.021	48.9**	165.4**	29.6**
	Samplings	5	2.92**	0.45	593	0.025	214.2**	74.7**	48.5**
	Plants × samplings	20	2.55**	0.33	294	0.010	31.3**	35.0**	17.1**
	Error between duplicates	30	0.0042				4.8	3.6	0.047
Dust-collector samples	Plants	3	14.09**	2.23**	11766**	0.168*	1300.4**	2962.5**	1975.0**
	Samplings	4	0.44*	0.40	1169	0.060	341.0**	770.5**	189.2**
	Plants × samplings	11	1.35**	0.36	1048	0.037	134.6**	214.8**	149.8**
	Error between duplicates	19	0.033				26.1	13.0	3.394

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

TABLE III

ANALYSES OF VARIANCE OF QUALITY IN EGG POWDERS, AS ASSESSED BY A FLAVOUR PANEL

Groups tested	Variance attributable to:	Degrees of freedom	Mean square
Prime quality samples	Plants	4	2.96
	Samplings	5	9.79**
	Plants × samplings	20	2.75
	Replicates	12	3.86*
	Error	335	1.92
Dust-collector samples	Plants	4	44.14**
	Samplings	3	19.55**
	Plants × samplings	12	12.23**
	Replicates	12	7.58**
	Error	200	2.64

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

Since palatability is a necessary judgment of desirability of foodstuffs, correlation of objective measurements with palatability is regarded as an important factor in the choice of methods. Analyses of covariance (Table IV) show that the two distinct sets of data, for prime quality and for dust-collector

TABLE IV

ANALYSES OF COVARIANCE OF PALATABILITY AND OTHER QUALITY MEASUREMENTS

Source of variance	Degrees of freedom	Mean square						
		Palatability and volatile materials	Palatability and bacterial count	Palatability and beating value	Palatability and pH	Palatability and water value	Palatability and KCl value	Palatability and fluorescence
Average regression	1	6.07**	1.34	35.20**	35.27**	32.03**	46.84**	49.20**
Differences in position	1	25.75**	29.60**	8.68**	3.56*	8.06**	0.81	0.53
Differences in slope	1	2.90*	0.38	1.00	4.91**	1.66	0.60	0.28
Residual	45	0.70	0.77	0.47	0.50	0.54	0.39	0.36

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

samples, may be combined for purposes of correlating palatability with fluorescence, and palatability with potassium chloride value. These two relations, therefore, are the only ones given in the general correlation table (Table V) under the grouping of prime quality and dust-collector samples.

Coefficients of correlation between the methods of assessing quality in dried egg powders are shown in Table V. On prime quality samples, beating value and pH were both mildly associated with potassium chloride value; pH was also correlated with fluorescence measurements. The slight degree of association between bacterial count and palatability was presumably fortuitous, since it was unconfirmed on the wider range of quality in dust-collector samples. However, all correlation values are low, and the general lack of correlation, considered in conjunction with palatability ratings, suggests that there was no great difference in quality between the prime samples.

The correlation coefficients between measurements made on residue material, from the secondary dust-collectors, showed that increase in moisture content was mildly associated with an increase in bacterial count. There is also some indication that a low moisture content was associated with a high fluorescence reading. Both these relations probably reflect plant practice. The six remaining measures of quality were all highly interrelated. All correlated to a somewhat higher degree with fluorescence values than with any other measurement, though an almost equal association was shown between palatability and potassium chloride value. Correlation of objective tests with palatability fell into three classes: moisture content and bacterial count

TABLE V

COEFFICIENTS OF CORRELATION BETWEEN VARIOUS QUALITY MEASUREMENTS

Groups tested	Methods	Methods						
		Volatile materials	Bacterial count	Beating value	pH	Water value	KCl value	Fluorescence
Prime quality samples	Bacterial count	.276						
	Beating value	−.038	−.030					
	pH	−.153	−.209	.127				
	Water value	.023	.113	−.177	.096			
	KCl value	−.130	−.086	.362*	.410*	.252		
	Fluorescence	−.125	−.327	−.254	−.498**	.186	−.229	
	Palatability	.170	.355*	.226	.024	.177	.248	−.310
Dust-collector samples	Bacterial count	.577*						
	Beating value	.293	.199					
	pH	.229	.025	.719**				
	Water value	.341	.103	.744**	.747**			
	KCl value	.406	.250	.834**	.726**	.800**		
	Fluorescence	−.511*	−.266	−.868**	−.851**	−.883**	−.913**	
	Palatability	.464	.266	.747**	.730**	.675**	.819**	−.854**
Prime quality and dust-collector samples	Palatability						.841**	−.866**

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

were not associated with palatability; correlations between palatability and beating value, pH, and water value all occupied an intermediate position; potassium chloride value and fluorescence measurements correlated most highly with palatability.

Discussion

Potassium chloride value and water value must not be confused with the results of soluble nitrogen determinations. Nitrogen values are concerned with protein only, operate at a much higher and narrower range of percentages, and were found to consume too much time and material to be of practical benefit in routine control work. Potassium chloride value and water value represent the amount of material passing through No. 1 Whatman filter paper. Since dried egg contains approximately 40% fat, ordinarily insoluble in water or potassium chloride solutions, the highest solubility to be expected in these tests would be about 60% of the original sample. In practice, however, higher values are observed, indicating the presence of egg oil in the filtrate. This behaviour can best be explained by the assumption that some combination of lipoid and protein, emulsion or otherwise, exists in the natural yolk, and carries the fat through the filter paper. Processing or storage treatments apparently alter this complex, resulting in low values by the procedures used. The range and sensitivity of the potassium chloride values obtained in this study lend considerable support to the above hypothesis, and suggest

that this empirical measurement may be more useful as a measure of quality than methods designed to determine the exact solubility of a particular constituent.

No correlation with palatability score was shown by any objective measurement on prime quality powders over the narrow range between 7.3 and 8.1 on the palatability scale. Of the methods studied here, the best objective measure of quality was fluorescence, correlating with palatability to the level of -0.854 on powders with a wide range of quality. However it is held that the difficulty on prime quality samples lies with the palatability test and not with fluorescence. Independent confirmation of this view is forthcoming from work now in progress in these laboratories. Very good powder has been lowered in quality, by a series of time–temperature treatments, to a level where a taste panel can distinguish the difference: within this range, fluorescence values establish a whole series of points, corresponding exactly with the time–temperature treatments. This indicates both the selectivity of the fluorescence method, and the insensitivity of panel ratings.

A more experienced panel may provide the more precise data necessary to establish correlation over the narrow high-quality range, and this possibility is being investigated.

Conclusions

Of the methods studied, moisture content, bacterial count, and one or more of the remaining tests are necessary for control purposes. Moisture content and bacterial count are independent measures of quality, contributing necessary information. Present evidence indicates that moisture content should be as low as is compatible with high quality in other respects. Bacterial counts yield information on the general condition of both the liquid egg and the powder produced. Of the remaining tests, fluorescence measurements and potassium chloride value are considered to be the most suitable, as they were more sensitive to minor differences in quality, and were associated with palatability to a closer extent than were the other methods studied. On the bases of selectivity and ease of operation, fluorescence is indicated as being the best single choice. However, since the correlation coefficients between potassium chloride value and palatability, and between fluorescence measurements and palatability did not differ significantly, the potassium chloride value may be useful if a fluorometer is not available.

Acknowledgments

The authors wish to express their appreciation of the assistance rendered by Mr. W. Harold White, Biochemist, and Dr. J. W. Hopkins, Statistician.

References

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 5th ed. A.O.A.C., Washington, D.C. 1940.
2. Pearce, J. A. and Thistle, M. W. Can. J. Research, D, 20(9) : 276-282. 1942.
3. Wilson, R. V. and Slosberg, H. M. Food Industries, 14(9) : 56-58. 1942.

THE EFFECT OF FREEZING AND THAWING ON THE QUALITY OF CANNED HERRING[1]

By F. Charnley[2] and O. C. Young[3]

Abstract

The effect of freezing and thawing on the quality of canned herring is investigated by comparing averages of the quality characteristics: firmness, volume of free aqueous liquid, and volume of free oil of treated samples with those of untreated samples. Repeated freezing and thawing definitely impairs the quality of canned herring by causing absorption of an appreciable proportion of the free aqueous liquid and free oil into the interior of the sample and seriously diminishing the firmness of the cooked tissue.

The investigation reported in this paper was undertaken to determine the magnitude of the changes in quality of canned herring arising through repeated freezing and thawing treatments. During the winter months, parcels of British Columbia canned herring that are shipped across Canada may, under certain circumstances, be subjected to one or more freezing and thawing cycles. Such a process, if accompanied by large temperature changes, will manifestly affect the quality of the canned product, but the extent of such changes in quality of canned products of this type does not appear to have been investigated hitherto. Accordingly, before it could be decided whether or not shipments of canned herring should be made in protected cars, it was necessary to ascertain the magnitudes of these changes in quality, as indicated by several easily measured quality characteristics of canned herring.

Treatment of Samples

The general plan of the experiments was very simple, namely, to submit samples of canned herring to several different freezing and thawing cycles and to compare the averages of the quality characteristics of the treated samples with those of untreated samples. In carrying out the experiments, however, it was found necessary to take into account three complicating factors. The first of these was that the quality characteristics of canned herring show considerable variation from sample to sample. Secondly, certain characteristics of this product are subject to pronounced seasonal variations, and, thirdly, it was necessary to allow for the effect of handling on the quality of the samples in the shipment of the experimental samples from Vancouver, B.C., to the Fisheries Experimental Station, Prince Rupert, B.C., and thence back again to the Canned Fish Inspection Laboratory, Vancouver, B.C.

The effect of the first and second of these factors was overcome by subjecting samples of 12 cans to the different treatments and comparing the

[1] *Manuscript received in original form April 7, 1942, and as revised, August 3, 1942. Contribution from the Canned Fish Inspection Laboratory, Vancouver, B.C., and the Pacific Fisheries Experimental Station, Prince Rupert, B.C.*

[2] *Chief Chemist, Canned Fish Inspection Laboratory, Vancouver, B.C.*

[3] *Associate Research Engineer, Pacific Fisheries Experimental Station, Prince Rupert, B.C.*

treated samples with a control sample of 12 cans of the same code, that is, with samples packed by the same cannery on the same day, while possible differences arising from the third factor were avoided by shipping the control samples, along with the samples to be treated, to the Fisheries Experimental Station, where the freezing and thawing experiments were conducted, and likewise returning the control samples along with the treated samples to the Canned Fish Inspection Laboratory, where the samples were tested and the changes in quality determined.

The experimental freezing and thawing cycles to which the samples were subjected were divided into three different treatments, samples of 12 1-lb. ovals and 12 1-lb. talls being subjected to each of the three treatments. These treatments were as follows.

(A). The samples were placed in an environment of approximately minus 22° C. and were removed 23 hr. later, when the centres of the cans had reached a temperature of minus 19° C. as determined by means of an automatic recorder and thermocouples inserted in additional 1-lb. oval and 1-lb. tall cans. The two sets of samples were then removed from the refrigerated chamber and allowed to stand in the basement of the building at a temperature of approximately 15° C. After about 15 hr. the temperature of the centres of the cans had risen to 14° C. The samples were then subjected to a second freezing cycle under the same conditions as the first for a period of about 28 hr., when the centres of these cans, as determined by thermocouples in adjacent cans, had reached a temperature of minus 22° C. The samples were again transferred to the basement of the building and attained the temperature of this room (15° C.) slightly over 17 hr. later. At the completion of the experiment on December 1 the samples were packed in shipping cases and forwarded to Vancouver, B.C.

(B). The samples were subjected to two freezing and thawing cycles as in (A) except that the first freezing was to a temperature of minus 16° C., the first thawing to 14° C., and the second freezing to minus 14° C.

(C). The samples were subjected to two freezing and thawing cycles as in (A) except that the freezing was to minus 10° C. in each case and the thawing was to 14° C.

The control samples consisting of 12 1-lb. oval and 12 1-lb. tall cans were stored in the basement of the building during the experiments and, as mentioned above, were returned under the same shipping conditions as the treated samples.

The three rates of cooling were arbitrarily chosen, since it was not known through what conditions a transcontinental shipment of herring might pass in midwinter. In any type of railway car that might be employed, the freezing would certainly be slow, and for those cans in the centre of the load the cooling would obviously be slower than for those on the outside. Consequently, in these experiments different rates and degrees of cooling were employed to ascertain whether or not these variables were significant in producing changes

in the quality of the canned product. In all three treatments the cans were merely placed in still air, the temperature of which was controlled.

Freezing and thawing curves corresponding to the three different treatments are shown in Fig. 1. These curves were contracted from the recorder charts, upon which the temperature at the centre of any particular can was recorded every eight minutes, and illustrate graphically the relations between the temperature of the centres of 1-lb. tall cans and the time in hours from the commencement of the experiment. As will be observed from the figure, the time of cooling in the first cycle was approximately 23 hr. and the time of warming was about 15 hr., while the cooling and warming periods in the second cycle were respectively 28 and 17 hr.

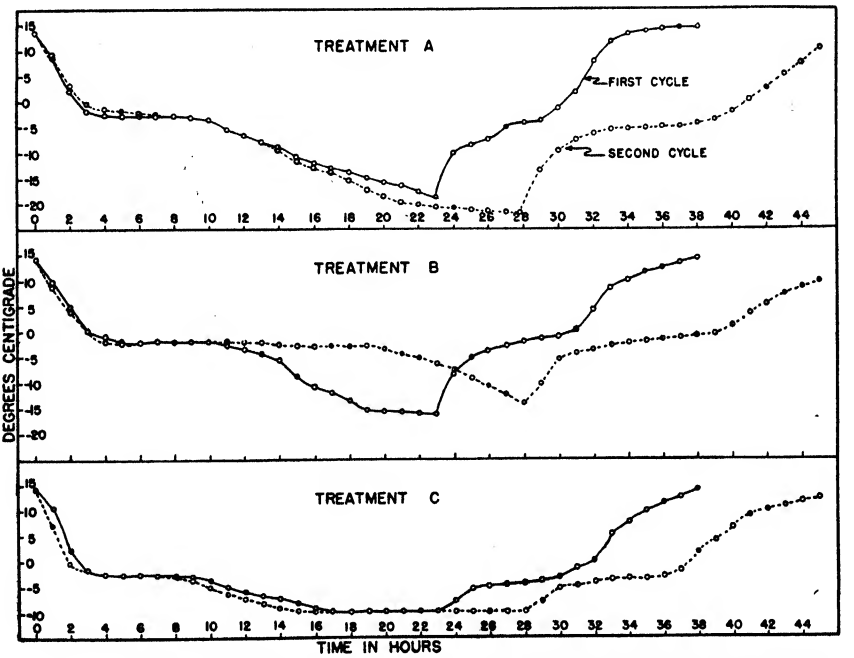


FIG. 1. *Freezing and thawing curves for 1-lb. tall cans of herring subjected to three different treatments.*

Examination of Samples

Changes in the quality of the samples resulting from the freezing and thawing treatments were investigated by measuring the following quality characteristics on individual samples: firmness of the fish tissue, volume of free aqueous liquid, volume of free oil, pH of free aqueous liquid, and refractive index of oil at 25° C. The last two of these characteristics, however, that is, the pH of the aqueous liquid and the refractive index of the oil, were not appreciably affected by the freezing and thawing treatments, possibly because, on the one hand, the addition of tomato sauce to the samples masked the

true pH values, and on the other hand, the free oil represents only a part of the total fat content of the samples, and would, furthermore, tend to exist in contact with only a portion of the surface of the solid tissue. Quality changes due to the freezing and thawing treatments were thus more satisfactorily determined by means of the first three characteristics.

In measuring firmness, values of the depth of penetration shown by an Armstrong penetrometer (1) were first obtained by taking averages of three readings on 1-lb. oval samples and single readings on 1-lb. tall samples. The values of depth of penetration were then converted into measures of firmness by means of the data (2) given in Table I. The advantages of the latter transformation are: *(a)* the resulting measure of firmness is a measure of the resistance of the sample to penetration; *(b)* the firmness increases in numerical value as the quality with respect to this characteristic improves; and *(c)* the measure of firmness is normally distributed, in contrast with depth of penetration which is highly skewed and leptokurtic (1).

TABLE I

CORRESPONDING VALUES OF MEASURE OF FIRMNESS AND DEPTH OF PENETRATION AT FIVE SECONDS

Depth of penetration, mm.	Firmness	Depth of penetration, mm.	Firmness	Depth of penetration, mm.	Firmness
4	24.9	12	7.8	20	3.7
5	21.0	13	6.9	21	3.4
6	17.9	14	6.2	22	3.2
7	15.3	15	5.6	23	3.0
8	13.2	16	5.1	24	2.9
9	11.4	17	4.7	25	2.7
10	10.0	18	4.3	30	2.1
11	8.8	19	4.0	35	1.7

The volumes of free aqueous liquid and free oil were measured by allowing the sample to drain for 15 or 20 min. into a graduate cylinder and reading off the respective volumes of the settled liquids at room temperature.

Typical results for the three characteristics, firmness, volume of free aqueous liquid, and volume of free oil are shown in Tables II and III, which give the results of the tests on the 1-lb. oval herring samples subjected to treatment B and on the 1-lb. oval samples used as controls.

Analysis of Results

Since there were three freezing and thawing treatments and two can sizes, the total number of measurements available for the estimation of the variance of each quality characteristic corresponding to a given can size was 48. A

TABLE II

RESULTS OF TESTS ON 1-LB. OVAL HERRING SAMPLES SUBJECTED TO TREATMENT B

Can No.	Depth of penetration, mm.	Vol. of free aq. liquid, cc.	Vol. of free oil, cc.	Can No.	Depth of penetration, mm.	Vol. of free aq. liquid, cc.	Vol. of free oil, cc.
1	11.0	23	3	7	16.3	18	3
2	11.7	8	1.5	8	18.0	14	4
3	11.7	5	3	9	14.7	14	3
4	13.0	0	1	10	13.7	18	3
5	16.0	17	5	11	13.3	10	1.5
6	12.3	16	4	12	12.3	26	7

TABLE III

RESULTS OF TESTS ON 1-LB. OVAL HERRING SAMPLES USED AS CONTROLS

Can No.	Depth of penetration, mm.	Vol. of free aq. liquid, cc.	Vol. of free oil, cc.	Can No.	Depth of penetration, mm.	Vol. of free aq. liquid, cc.	Vol. of free oil, cc.
1	10.7	14	3	7	9.7	22	5
2	14.7	13.5	3	8	12.7	17	2.5
3	13.3	21.5	4	9	15.0	23	4.5
4	11.3	29	3.5	10	13.0	13.5	3
5	12.3	32.5	6	11	11.0	19	3.5
6	12.0	26	3.5	12	11.3	19	7

simple and, at the same time, flexible method of evaluating the results, therefore, was to estimate variances from the pooled results for each can size and to determine the significance of the differences in means by converting the latter into t values. Where necessary, it was then a relatively simple matter by utilizing the fact that the differences in freezing and thawing treatment resulted in no observable differences in quality to increase the sensitiveness of the comparisons by combining the results derived from the treated samples.

Tables IV to IX give the details of the estimation of the variances of the individual quality characteristics corresponding to given can sizes, together with the t values corresponding to the differences in means between the treated and control samples. Application of Pearson's formula (4), namely,

$$\sigma_e^2 = \frac{1}{N - k} \sum_{i=1}^{k} (n_i s_i^2)$$

to the totals of the columns headed "ns^2" gives the estimates of the variances of the individual quality characteristics shown at the bottom of Tables IV to IX. The variance of the difference in two averages of 12 (σ_d^2) required in calculating the t values is evidently 1/6 of the estimated variance given in the tables.

TABLE IV

ESTIMATION OF VARIANCE OF FIRMNESS OF 1-LB. OVAL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	8.1622	5.8750	−1.8667	−3.9827
B	12	21.7565	6.6833	−1.0584	−2.2582
C	12	6.1491	5.4917	−2.2500	−4.8005
Control	12	21.9691	7.7417		
Total	48	58.0370		$\sigma_e^2 = 1.3190$	$\sigma_d = 0.4687$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE V

ESTIMATION OF VARIANCE OF VOLUME OF FREE AQUEOUS LIQUID OF 1-LB. OVAL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	430.06	17.1250	−3.7083	−1.4616
B	12	598.92	14.0833	−6.7500	−2.6605
C	12	258.73	14.7917	−6.0416	−2.3813
Control	12	411.67	20.8333		
Total	48	1699.38		$\sigma_e^2 = 38.622$	$\sigma_d = 2.5371$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE VI

ESTIMATION OF VARIANCE OF VOLUME OF FREE OIL OF 1-LB. OVAL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	6.562	3.3750	−0.6667	−1.3933
B	12	29.750	3.2500	−0.7917	−1.6545
C	12	3.916	2.9167	−1.1250	−2.3511
Control	12	20.229	4.0417		
Total	48	60.457		$\sigma_e^2 = 1.3740$	$\sigma_d = 0.4785$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE VII

ESTIMATION OF VARIANCE OF FIRMNESS OF 1-LB. TALL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	77.8860	4.2333	−2.3000	−3.1949
B	12	23.9622	4.4250	−2.1083	−2.9286
C	12	13.3621	4.8250	−1.7083	−2.3730
Control	12	21.6065	6.5333		
Total	48	136.8168		$\sigma_e^2 = 3.1095$	$\sigma_d = 0.7199$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE VIII

ESTIMATION OF VARIANCE OF VOLUME OF FREE AQUEOUS LIQUID OF 1-LB. TALL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	1793.23	38.2917	−13.375	−2.8058
B	12	969.42	31.4167	−20.250	−4.2480
C	12	1099.67	41.1667	−10.500	−2.2027
Control	12	2136.67	51.6667		
Total	48	5998.99		$\sigma_e^2 = 136.340$	$\sigma_d = 4.7669$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE IX

ESTIMATION OF VARIANCE OF VOLUME OF FREE OIL OF 1-LB. TALL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	61.062	11.3750	−5.1250	−5.1204
B	12	74.729	11.2917	−5.2083	−5.2036
C	12	59.667	10.8333	−5.6667	−5.6616
Control	12	69.000	16.5000		
Total	48	264.458		$\sigma_e^2 = 6.0104$	$\sigma_d = 1.0009$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

From an inspection of Tables VII, VIII, and IX, it will be seen that the 1-lb. tall herring samples, which were subjected to the freezing and thawing treatments, differ very significantly from the control samples as regards firmness, volume of free aqueous liquid, and volume of free oil, and, as shown in Table IV, there are similar significant differences in firmness between the treated and control 1-lb. oval herring samples. In fact, in these four sets of data the differences in the averages are so pronounced that it is not necessary to combine the results derived from the treated samples to demonstrate the presence of significant effects. The lowest t value (2.2027) in these four sets would occur by chance less than four times in 100, while t values approximating 2.5 and 3 are associated with probabilities that are less than 0.02 and 0.01 respectively. There is thus no doubt in these four sets of data of very definite changes in quality due to the freezing and thawing treatments.

In contrast with the results of the 1-lb. tall samples, the t values of Tables V and VI corresponding to differences in the free aqueous liquid and free oil of the 1-lb. oval samples are not all individually significant. The t values associated with treatments B and C in Table V, and treatment C in Table VI are individually significant, but the remaining values are not individually conclusive. The fact that the values are all of the same sign, however, readily suggests that significant differences would be obtained on comparing the results of the tests on the control samples with the combined results derived from the treated samples.

This inference is found to be correct. Comparison of the variance between treated samples with the variance within samples, as summarized in Tables X and XI, shows that the treated samples may safely be considered homogeneous as regards the characteristics, free aqueous liquid and free oil. The values of z are, respectively, -0.1199 and -0.3562 each with $n_1 = 44$ and $n_2 = 2$ degrees of freedom. The 5% point, however, for $n_1 = \infty$ and $n_2 = 2$ is, from Fisher's (3) table, $z = 1.4851$, so that, as judged by these data, there is no evidence of lack of homogeneity in the samples, and consequently, no evidence of any difference in quality resulting from the differences in freezing and thawing treatment.

TABLE X

ANALYSIS OF VARIANCE OF FREE AQUEOUS LIQUID IN 1-LB. OVAL HERRING SAMPLES

Variance	D.f.	Sum of squares	Mean square	Log_e s.d.
Between treated and control samples	1	272.25	272.25	2.8034
Between treated samples	2	60.79	30.39	1.7070
Within samples	44	1699.38	38.62	1.8269
Total	47	2032.42		

TABLE XI

ANALYSIS OF VARIANCE OF FREE OIL IN 1-LB. OVAL HERRING SAMPLES

Variance	D.f.	Sum of squares	Mean Square	Log. s.d.
Between treated and control samples	1	6.674	6.674	0.9491
Between treated samples	2	1.347	0.674	−0.1973
Within samples	44	60.457	1.374	0.1589
Total	47	68.478		

Accordingly, we may legitimately combine the treated samples and compare the averages of the combined results with the averages given by the control samples. From Table V the average free aqueous liquid for the treated samples is 15.3333. The difference in average free aqueous liquid in the treated and control samples is therefore 5.5000 with variance 1/9 of the estimated variance. Hence the value of t is 2.654 with 44 degrees of freedom. Similarly, the corresponding value of t for the data of Table VI is 2.203 with the same number of degrees of freedom. Consequently, the differences between the treated and control 1-lb. oval herring samples with respect to volume of free aqueous liquid and volume of free oil are also significant.

From an examination of the data listed in Tables IV to IX, it will be observed that the variances of the treated samples differ considerably in a number of treatments from those of the control samples. The variances corresponding to treatments A and C of Tables IV and VI, and treatment B of Table VIII for example, are apparently much lower than the corresponding variances in the control samples, and the reverse is true of the variances of treatment A of Table VII. It could therefore be contended that the resulting t values have been largely influenced by the differences in the variances and that, as a consequence of this, the differences in the means are not necessarily significant.

That the means themselves differ significantly, however, is readily demonstrated by taking advantage of the fact that all the differences are of the same sign and applying a test appropriate to this circumstance. If, for example, the control samples of the 1-lb. oval herring data are segregated into groups of four and each of these groups is associated with a corresponding treatment, the averages shown in Table XII are obtained. From these results it is seen that the differences in the averages between the treated and control samples are in all except one instance negative, and in the one exception the difference is zero. Since, by virtue of the method of packing, all these characteristics are substantially independent, it follows that, even if the magnitudes of the differences in the averages are neglected, the probability of obtaining the eight minus signs is $(1/2)^8 = \frac{1}{256}$ under the hypothesis that the means do not differ significantly. There is thus no doubt that the freezing and thawing treatments have definitely impaired the quality of these samples.

TABLE XII

COMPARISON OF AVERAGES OF FOUR CONTROL SAMPLES WITH AVERAGES OF TREATED 1-LB. OVAL HERRING SAMPLES

Treatment	Firmness		Free aqueous liquid		Free oil	
	Treated	Control (Av. of 4)	Treated	Control (Av. of 4)	Treated	Control (Av. of 4)
A	5.875	7.550	17.125	19.500	3.375	3.375
B	6.683	8.225	14.083	24.375	3.230	4.250
C	5.492	7.450	14.792	18.625	2.917	4.500

The fact that the volume of free aqueous liquid and volume of free oil of the 1-lb. oval herring samples are relatively less affected by the freezing and thawing treatments than these characteristics of the 1-lb. tall herring samples is apparently connected with the difference in the methods of processing the two can sizes. In packing oval cans part of the liquids formed is usually drained off, whereas these liquids are usually retained in packing 1-lb. tall cans.

Conclusion

From the foregoing results it must, therefore, be concluded that repeated freezing and thawing definitely impairs the quality of canned herring. Freezing and thawing of this product apparently causes considerable changes in the fish muscle tissue with absorption of an appreciable proportion of the free aqueous liquid and free oil into the interior of the sample, thus resulting in a serious diminution in the firmness of the cooked tissue.

Acknowledgments

The writers acknowledge with thanks the valuable assistance rendered by other members of the staffs of the Pacific Fisheries Experimental Station and the Canned Fish Inspection Laboratory in carrying out the above investigation. To Dr. N. M. Carter, Director, Mr. E. P. Sidaway, Scientific Assistant in Research Engineering, and Mr. Geo. Baker, Junior Technician, of the Fisheries Experimental Station, thanks are due for very substantial assistance in carrying out the freezing and thawing experiments, while similar thanks are extended to Mr. R. S. Bolton, Mr. F. R. E. Davies, Miss P. C. Cumming, and Mrs. O. Volkoff, Assistants at the Canned Fish Inspection Laboratory, for their work in carrying out the tests on the experimental samples, and also a number of the calculations in the preparation of the paper. Finally, sincere thanks are due to the reviewers of the manuscript for a number of very helpful criticisms in the statistical treatment of the data and abbreviation of the tables.

References

1. CHARNLEY, F. and BOLTON, R. S. J. Fisheries Research Board Can. 4(3) : 162-173. 1938.
2. CHARNLEY, F. and BOLTON, R. S. Unpublished results.
3. FISHER, R. A. Statistical methods for research workers. 5th ed. rev. and enl. Oliver and Boyd, Ltd., Edinburgh and London. 1934.
4. PEARSON, E. S. J. Roy. Stat. Soc. (n.s.) 96(1) : 21-60; discussion, 60-75. 1933.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. FEBRUARY, 1943 NUMBER 2

GROWTH OF THE SALMON EMBRYO[1]

BY F. R. HAYES[2] AND F. H. ARMSTRONG[3]

Abstract

Wet and dry weights of Atlantic salmon are given up to the end of yolk sac absorption, and from them the growth rates are determined. Attempts are made to smooth the growth curve by the methods of Brody, Murray-Schmalhausen, and MacDowell *et al.* Of these the last is best taking zero time as nine days after fertilization. It is concluded that, as to weight, the interval considered ends before the point of inflection of a Sachs growth cycle. Growth in length, however, represents a complete cycle, hence there can be no simple quantitative relation between length and weight. Deviations from the smoothly descending relative growth rate (RGR or Minot) curve are considered, with the conclusion that all such irregularities so far presented can be attributed to random errors (except possibly the posthatching rise in RGR of the trout at 12° reported by Wood). In general weighing is not sufficiently sensitive as a method, to permit a detailed description of the RGR.

The purpose of this paper is to interpret a series of weights of developing embryos of the Atlantic salmon, *Salmo salar* L., taken from as soon after fertilization as possible up to the time of complete absorption of the yolk sac. Groups of 10 embryos, dissected off the yolk sac, were placed in tared Pregl micro weighing bottles and weighed to the nearest 0.2 mg. to obtain the "wet weight"; they were then dried at 100° C. to a constant value called the "dry weight". Five to 10 such groups were used in order to obtain each of the figures given in Table I, Columns 3 and 4—thus each value is based on 50 to 100 embryos. In all about 2000 embryos were used. Further details of technique, including the use of formalin for fixation of early stages, are given elsewhere (9).

It will be convenient to examine the salmon values in relation to the classical ideas of growth, and this will be facilitated by a brief preliminary description of a generalized growth cycle and the chief curves derived from it. Consideration will also be given to some of the formulae that have been put forward for the purpose of smoothing embryonic growth curves.

A Growth Cycle and Its Derivatives

If one measures the weight or length of a growing organism at various intervals of time, any two determinations differ by amounts somehow related

[1] *Manuscript received August 12, 1942.*

Contribution from the Zoological Laboratory, Dalhousie University, Halifax, N.S. With financial assistance from the National Committee on Fish Culture.

[2] *Associate Professor of Zoology.*

[3] *Student Assistant in Zoology.*

to the time that has elapsed between them. If time is plotted on the abscissa and weight or length on the ordinate the resultant curve is often more or less *S*-shaped (Fig. 1A). Sachs (22) called the data described by the curve, a growth cycle or great period of growth. Such a curve begins at or near the zero point and is at first convex to the abscissa, later concave, so that somewhere in the middle there must be an inflection or change of direction of

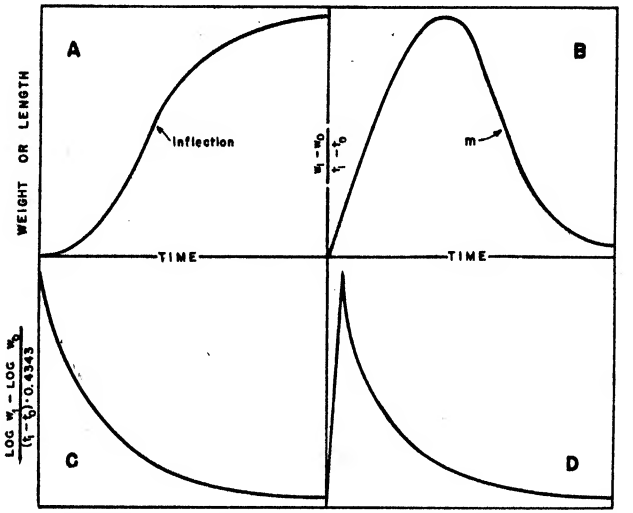


FIG. 1. *A growth cycle and its derivatives. A. Complete cycle of Sachs. B. Absolute growth rate. C. Usual relative growth rate or Minot curve. D. Occasional RGR as in a few plants.*

curvature. The curve is characteristically asymptotic to the final size, i.e. it never really becomes flat at the top, which means in theory that an organism never stops growing, although up at the top of the *S* the rate of increase is so small that the slope ceases to be of interest. Although a growth cycle, mathematically, extends to infinity, in practice there is often a more or less clearly marked end, e.g. the onset of a second cycle or the withering of a plant; it is with reference to such an end that expressions like "centre of a cycle" are used in the following pages.

Growth cycles may be observed when the environmental conditions are kept approximately constant, as in Sachs' classical experiment on the increase in length of the bean during a few weeks in summer. They may also be observed, in spite of fluctuating conditions, when the unit of measurement is the year, for under such circumstances seasonal variations cancel themselves out, e.g. Pütter's observations on length growth of the herring, as cited by Backman (2). On the other hand no *S*-shaped curve would be expected, for example, in the growth of an apple bud, which begins in one summer, is suppressed during the winter, and goes on in the spring to become a blossom or leaf. The same is true of the discontinuous development of insect larvae.

Moreover there can be no general growth cycle for a species in which the individuals differ in some essential habit; for example the Atlantic salmon may go to sea in its second or third year, return in its fourth or some later year, and may or may not return to the river a second or third time.

Some of the properties implicit in an *S*-curve can be brought into view by changing it around to show absolute growth rate (or AGR) (Fig. 1B), which answers the question "How much is added per unit time to what is already there?" The AGR is obtained by dividing the increase in weight by the increase in time, or

$$AGR = \frac{dw}{dt} = \frac{w_1 - w_0}{t_1 - t_0}$$

approximately, where w_0 is the weight at any time, t_0, and w_1 is the weight at a slightly later time, t_1. The curve passes through a maximum or peak, to the right of which there is an inflection, m. The presence of m is a mathematical necessity. It is mathematically possible, but not necessary, for a second inflection to occur to the left of the peak, more or less opposite to m. (But data that can be reduced to a straight line by the method of MacDowell *et al.* described below, cannot exhibit any second inflection in the AGR curve.) The AGR curve should begin at the origin, i.e. have a zero value at zero time. Unfortunately biological zero time is difficult to determine, being reckoned sometimes from fertilization, sometimes from the first formation of the embryonic axis, and sometimes from a mathematically convenient point without biological meaning. Thus the theoretical requirement is not always realized.

In 1891 Minot suggested that the growth rate should be expressed in terms of the material already there by use of the formula:

$$\text{Percentage growth rate (Minot)} = \frac{100\ (w_1 - w_0)}{w_0\ (t_1 - t_0)}.$$

Schmalhausen (23) and Brody (4) have independently shown that the Minot formula tends to give results that are too high, especially in the youngest stages, and have proposed to substitute for it

$$\text{Relative growth rate} = \underset{\text{(Schmalhausen)}}{C_v} = \underset{\text{(Brody)}}{k_1} = \frac{dw}{w.dt} = \frac{\log w_1 - \log w_0}{(t_1 - t_0).0.4343}.$$

C_v (or Brody's k_1) has been used for the calculations of relative growth rate in Table I. It is a better version of $\frac{\text{Minot}}{100}$. ("Log" throughout this paper refers to common logarithms to the base 10.)

Fig. 1, C and D shows typical relative growth rate or Minot curves. In a few plants the curve may have the same form and properties as the absolute growth rate curve, but the peak is far to the left (Fig. 1D). An example is found in Silberschmidt's work, cited by Backman (2, p. 925), on the growth of the oat at 4° C. Such curves are special cases, for in nearly all plants, and in all animals so far investigated (except for Privolniev's (19) salmon

TABLE I

WEIGHTS ARE OF ONE EMBRYO IN MILLIGRAMS. TO OBTAIN COLUMN 5 EACH WEIGHT READING IN COLUMN 4 WAS AVERAGED WITH THOSE BEFORE AND AFTER IT. THE SAME WAS DONE WITH COLUMN 2 TO OBTAIN THE TIMES GIVEN IN COLUMN 7

1	2	3	4	5	6	7	8	9	10	11
Days before and after hatching	Days after fertilization	Dry weight	Wet weight	Averaged wet weight	Log Col. 5	Averaged days for Col. 5	Central dates from Col. 7	Absolute growth rate from Cols. 5 and 7	C_v or relative growth rate from Cols. 6 and 7	$C_t\,(t-9)$ from Cols. 8 and 10
−40	10		.16	.16	.2041	10				
							10.5	.15	.66	1.0
−39	11		.31	.31	.4914	11				
							11.5	.34	.74	1.8
−38	12		.45	.65	.8129	12				
							13.0	.23	.26	1.1
−36	14	.19	1.2	1.1	.0414	14				
							15.5	.20	.15	1.0
−33	17	.25	1.6	1.7	.2304	17				
							18.5	.50	.21	2.0
−31	19	.35	2.2	3.2	.5051	20				
							21.5	.70	.17	2.1
−26	24	.5	5.8	5.3	.7243	23				
							25.0	.60	.094	1.5
−23	27	.8	7.8	7.7	.8865	27				
							28.5	.47	.056	1.1
−19	31	.9	9.4	9.1	.9590	30				
							31.0	.75	.076	1.7
−18	32	1.0	10.2	10.6	.0253	32				
							33.0	.60	.054	1.3
−17	33	1.3	12.3	11.8	.0719	34				
							35.5	.80	.062	1.6
−14	36	1.5	13.0	14.2	.1523	37				
							39.0	.65	.042	1.3
− 9	41	2.0	17.4	16.8	.2253	41				
							43.0	.80	.044	1.5
− 3	47	2.4	20.0	20.0	.3010	45				
							47.0	.70	.033	1.3
− 2	48	3.1	22.6	22.8	.3579	49				
							51.0	.62	.026	1.1
3	53	3.4	25.8	25.3	.4031	53				
							56.5	.74	.027	1.3
7	57	3.8	27.5	30.5	.4843	60				
							63.5	.99	.029	1.6
19	69	5.8	38.2	37.4	.5729	67				
							71.0	.86	.021	1.3
26	76	7.4	46.5	44.3	.6464	75				
							77.5	.92	.020	1.4
30	80	7.7	48.1	48.9	.6893	80				
							82.0	.95	.019	1.4
33	83	8.3	52.2	52.7	.7218	84				
							86.5	1.7	.030	2.3
38	88	9.1	57.7	61.3	.7875	89				
							95.0	1.3	.019	1.6
47	97	12.8	73.9	77.1	.8871	101				
							109.5	1.3	.015	1.5
68	118		99.6	99.6	.9983	118				

embryo values discussed below), the curve starts at a maximum and falls continuously, approaching the time axis asymptotically (Fig. 1C). Whether it has bumps on it representing fluctuating growth rates, and if so how many, remains an open question.

Irregularities in Growth Rate Curves

It is true of growth rate curves more than any other under consideration, that there have to be many closely spaced observations for an accurate representation of the facts. As Figs. 3 and 6A show, the points fluctuate a good deal, thereby increasing the difficulty of smoothing the curve by eye.

The cause lies in the arithmetic, for it will be noticed in the absolute growth rate (and the same reasoning would apply to the relative growth rate),

$$AGR = \frac{\text{large weight minus slightly smaller weight}}{\text{large time minus slightly smaller time}}.$$

There are two chief sources of error. First, the numerator shows errors in weight estimations, which are relatively greater in younger stages when the embryos are very small; second, errors arise from the fact that even under identical conditions development does not proceed for all embryos at the same rate, which is equivalent to an error in the times given in the denominator. The latter may be relatively unchanged throughout development. If hatching, for instance, were to be placed at a certain date plus or minus one week, the end of yolk absorption might be taken at plus or minus two weeks or more. To illustrate specifically, taking letters to represent the relative errors,

$$AGR = \frac{(22.8 \pm p) - (20.0 \pm q)}{(49 \pm r) - (45 \pm s)}$$

$$= \frac{2.8 \pm (p + q)}{4 \pm (r + s)}.$$

This is approximately

$$AGR = 0.7 \times [1 \pm (8.1p + 7.1q + 12.3r + 11.3s)].$$

It is obvious that the calculation of a growth rate causes figures with small errors to be changed into a figure with a much larger error. Some caution is accordingly necessary in the matter of placing a physiological interpretation on the scattering of growth rate points, as Privolniev (19) has done.

The Growth Curve of the Salmon

Fig. 2 shows the wet and dry weights of the salmon embryo plotted against time (Table I, Columns 2, 3, and 4). The ordinates have been selected in such a way as to suggest that the dry weight rises relatively more rapidly than the wet weight, which is true, and which leads to the conclusion that the proportion of water in the embryo begins to decrease some time before hatching and becomes progressively less as development proceeds. Otherwise the curves are similar in form, and when they are compared to Fig. 1A it is immediately apparent that the point of inflection of the latter has not yet been reached by the salmon. Thus the salmon, up to the completion of its yolk sac absorption, represents less than half of a growth cycle. This important conclusion, which differs from that of some other authors, will be referred to again in the next section.

In the remainder of this paper only the wet weight values will be used for plotting curves, for the dry weights would probably add little to the conclusions to be drawn, and are moreover, incomplete at the beginning and end.

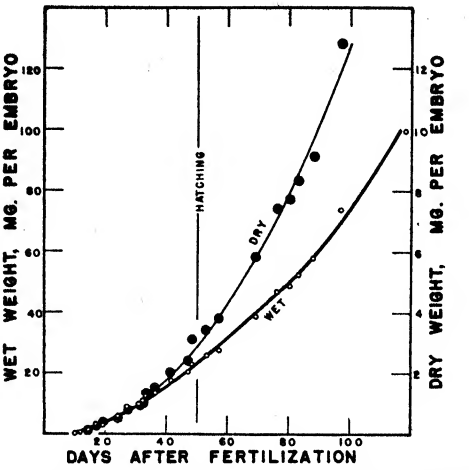


FIG. 2. *Growth of the salmon expressed in wet and dry weight.*

The Absolute Growth Rate

Because of the tendency for points on growth rate curves to fluctuate, it was considered advisable to carry out a preliminary smoothing before plotting the absolute growth rate. Each weight reading, and its corresponding time reading, was averaged with those before and after it, the new results being given in Table I, Columns 5 and 7. From these the AGR was calculated (Table I, Column 9, and Fig. 3). The method of getting Column 9 is equivalent to ignoring the two items of Column 4 nearest to the central date and using only the two items next further away. According to Thompson (26, p. 138) the method was first used by Gauss. Its advantage lies in the clarification of general trends, its disadvantage is a tendency to minimize or obliterate sudden changes which may be real.

If the prehatching part of the AGR curve be projected back to the abscissa it is found that the intersection falls at five days, a value for which no great accuracy can be claimed, and which might precede, or at latest coincide approximately with the formation of the embryonic axis which in the present observations occurred at about nine days. Thus there cannot be, after the embryo is formed, any sweeping concavity in the curve. It is more likely that the embryo starts off at a maximum AGR. However, other workers have reported an early inflection in AGR curves of trout (8 and 13), and salmon (19). Privolniev's early values are completely at variance with the present ones (see below); as to possible differences between the salmon and trout, no decision can be made at present.

An additional peculiarity of the salmon is the interruption of the curve at hatching, which is less unexpected in view of previously noted interruptions of particular embryonic activities by hatching (10). Whether there is

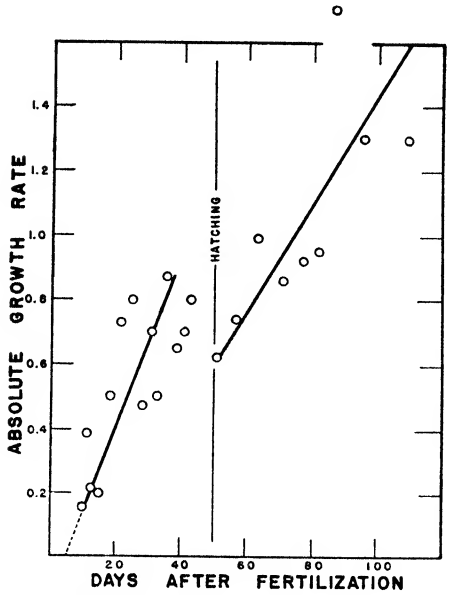


FIG. 3. *Absolute growth rate of the salmon, from wet weights.*

actually a dip in the AGR curve or merely a failure to continue the upward slope, is not certain. The difference in slope between the pre- and post-hatching curves is not significant. It may be doubted whether the hatching interruption occurs under all conditions, or whether there is not perhaps an optimum temperature at which development proceeds through hatching without a break, but above and below which hatching exercises an interfering action.

A third feature of the salmon curve (noted in the preceding section) is that the peak, as in Fig. 1B, has not yet been reached, from which it is concluded that the first winter of salmon life cannot be called a growth cycle in the sense of Sachs. Working on the trout, Wood (30) also failed to find a decline in AGR. This is not to deny that when the yolk is all gone, or nearly all gone, the embryo, owing to starvation, will necessarily stop growing. Gray (8) and Kronfeld and Scheminzky (13) reported for the trout and Privolniev (19) for the salmon that a decline in AGR begins before the yolk is quite gone. Differences may be in part due to the difficulty in deciding when the yolk is entirely used up, for there is still some left when the external sac has disappeared. If the reported decline in AGR could be abolished by feeding, the idea of a growth cycle would have to be abandoned; the experiment has been done with trout by Willer (29). He weighed whole larvae, hence his values represent embryo gain minus yolk loss, which would tend, if anything, to produce the effect of a decline after hatching even if none existed

for the embryo. Feeding was begun, in conformity to hatchery practice, when the yolk sac was partly absorbed, and there was in fact no sign of a decline in growth up to the time the fry were ready for planting, two months after the yolk sac had completely disappeared.

Smoothing the Salmon Curve

A simple and somewhat primitive method of smoothing the wet weight curve has already been described. Various workers have recommended other methods involving the use of algebraic expressions, some of which must be considered. It will not however, be possible to deal with formulae having the purpose of describing the whole *S*-curve, as in Fig. 1A, because the salmon does not, during the first winter of life, reach the point of inflection. Therefore knowledge is lacking as to the final weight attained and the total time for a cycle, which are required for the application of equations such as those of Robertson (21) and von Hoesslin (12). An objection to other of these formulae is that most of them have three arbitrary constants, being of the general form:

$$\log w = k_0 + k_1 (t) + k_2 (t)$$

where k_0 is the intercept, k_1 the slope, and k_2 the correction for the slope; (t) may mean variously $\log t$, t^2, $\log {}^2t$, etc. (3). It is extremely doubtful whether there are any embryonic weight data precise enough to warrant for their description the use of three arbitrary constants. Hence, the salmon weights cannot be profitably interpreted in terms of such expressions.

An alternative method of treatment, more desirable for present purposes, is to deal only with the part of a Sachs curve in which one is interested. The curve readily divides itself into two, or sometimes three parts (when the middle segment appears as a straight line). Of these only the first part, which is convex to the abscissa, parallels the salmon weight results. To expressions describing it the objection has been raised that they do not give reasonable results when the curves are projected either to zero time or to infinity, or both. Thus an embryo might, according to the formula, grow to infinite size. As it is not proposed to extrapolate the salmon results the objection need not be taken seriously. The purpose of curve smoothing is to force the graph into a straight line in order to permit easy interpolation.

The first expression to be considered is that of Brody (4). In working with embryonic chicks Brody was struck by the fact that when the log of the weight was plotted against time a series of straight lines resulted, and this led him to the view that the relative growth rate, k_1, remained constant for a time, then changed suddenly. During one of these constant periods the rate could be represented by the expression

$$\log w = k_0 + k_1 t.$$

When the slope changed there were new values for k_0 and k_1. Brody pays no attention to the k_0 or intercept values, which are wholly unreasonable, but is concerned entirely with the slope, k_1. In the upper part of Fig. 4,

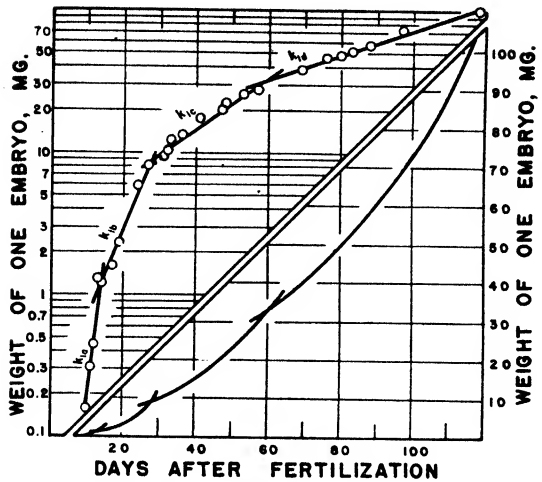


FIG. 4. *Upper part, salmon wet weights as smoothed by Brody's method in which weight is plotted on a logarithmic scale, time on an arithmetic scale. Lower part, a Brody interpretation of Fig. 2.*

by plotting the wet weights on a logarithmic scale, an attempt has been made to apply Brody's method to the salmon. In accordance with Brody's procedure the breaks have been selected arbitrarily. It has been possible to show by Fisher's method of analysis of variance, that the slopes of the lines differ significantly from each other. Except for the second segment, k_{1b}, it has not been possible to show with existing data, that the segments between the breaks are well fitted by straight lines. Additional ground for scepticism is provided by the lower part of the figure, which represents the original growth curve as smoothed from the Brody results. Considerably more evidence than is available would be required before the lower part of Fig. 4 could be accepted as a description of the data set down in Fig. 2. Nevertheless whatever may be the quantitative difficulties with Brody's method of presentation, it does direct attention to the idea that the growth rate may change abruptly.

While Brody considered the relative growth rate as changing at intervals from one constant value to another constant value, Friedenthal (6) and Murray (17) had, with similar data, come to a somewhat different conclusion, namely that when embryo weight is plotted against time on logarithmic paper (where the abscissa and ordinate are proportional to the logs of the numbers), the result is a straight line;

$$\log w = k_0 + k_1 \log t.$$

This is another way of saying that the corners of the segments in Brody's curve (lower part of Fig. 4) should be rounded off, and the whole represented

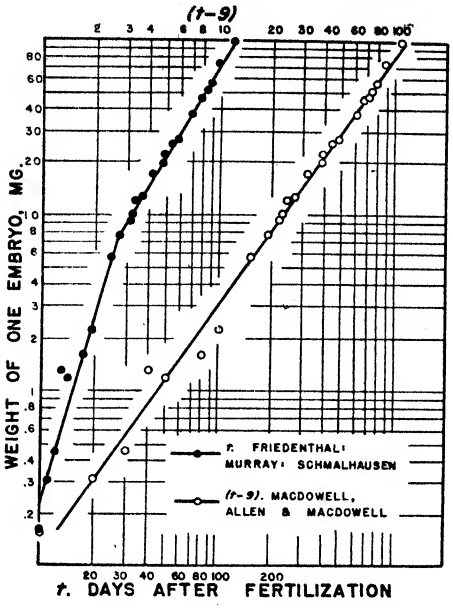


FIG. 5. *Data of Fig. 2 as changed by plotting on logarithmic paper. Left, when fertilization is taken as zero time. Right, when the formation of the embryo at nine days is taken as zero time.*

as a generalized parabola of the type $y = Ax^{k_1}$. Schmalhausen, although he started out from an entirely different point of view, arrived at the same conclusion (24). In Fig. 5 the Friedenthal-Murray-Schmalhausen equation is tested by a plot of the salmon data on logarithmic paper. It is apparent that the values cannot be fitted by one straight line, although they can be fitted by two straight lines with a break that corresponds to that between segments two and three of the Brody curve. (Note: Glaser's (7) modification of the above formula offers no improvement when applied to the salmon data.)

The final step in curve smoothing was taken by MacDowell, Allen, and MacDowell (15) who proposed to write $(t - n)$ instead of t in the preceding formula, where n is the number of days after fertilization at which the embryonic axis is established. In the present case n may be taken with fair probability as 9. The theory is that before n days when there is an undifferentiated mass of cells spreading over the yolk sac the rules of growth will not be the same as later, when the observer is dealing with the embryo proper. Hence time should be reckoned from the establishment of the permanent plan. While n is of course a new constant, it is a natural one, directly observable in the material, to whose use no objection can be taken. In Fig. 5, $(t - 9)$ has been plotted against w on logarithmic paper, as a test of the formula

$$\log w = k_0 + k_1 \log (t - 9).$$

The points can be fitted by one straight line, which means that the salmon

data have been smoothed in a systematic way according to a very simple plan. The MacDowell formula has been successfully applied, by proper choice of n to a considerable variety of embryonic data. The time before n is necessarily considered as irrelevant, for trouble would arise if log $(t - n)$ were applied when t is less than n, as negative numbers do not have real logs.

Brody's formula directs attention to sudden change; MacDowell's formula suppresses evidence of sudden change. The views are not inconsistent, any more than a description of the earth as round contradicts knowledge of mountain heights and ocean depths. On the causes of irregular variation in salmon growth rate, if such exist, the evidence is still inadequate. But even where full details are available about the extent and nature of a fluctuating growth rate, those who prefer a single, comprehensive formula are willing to apply it to the results. Thus Courtis (5) finds that the growth of the City of Detroit can be treated as a Sachs cycle and fitted by the three-constant formula of Gompertz, with an average deviation of only 5% of the mean "omitting the ten depression years: 1830 and 1834 following the War of 1812; 1860, 1864, and 1870, the Civil War depression: and 1930, 1932, 1934, 1935, 1936, the World War depression years. The A. D. for these years was 38%. Three other large errors occurred between 1840 and 1850."

The Relative or Percentage Growth Rate

If C_v be plotted against time, a Minot curve should result, although the latitude of interpretation discussed in the preceding section will have a corresponding effect on its shape. It is well to begin with a comparatively unsmoothed curve, Fig. 6A, which was constructed from Table I, Column 10. The irregularities in the present salmon values appear to be random, and provide no evidence of systematic deviation from a smoothly descending curve. In fact the curve is like that for warm-blooded vertebrate embryos (18), and does not justify the view that fish embryos differ in their growth mechanism from birds and mammals.

Conformation of the similarity is provided by the values in Table I, Column 11, for according to Schmalhausen's "law of growth" (24) the product of the multiplication of the relative growth rate by the age is a constant,

$$C_v t = K.$$

This is just another way of saying that log weight plotted against log time gives a straight line (MacDowell *et al.* curve). Schmalhausen's table (reproduced in 18, p. 436) shows that for warm-blooded animals the constant is usually between three and four. The only fish embryo included is the trout for which $C_v t = 2.06$ (data of Kronfeld and Scheminzky). Time is reckoned from the establishment of the embryonic axis, 10 days in the trout. Under present conditions time is taken as $(t - 9)$, and on the average of Table I, Column 11,

$$C_v (t - 9) = 1.47 \pm 0.05.$$

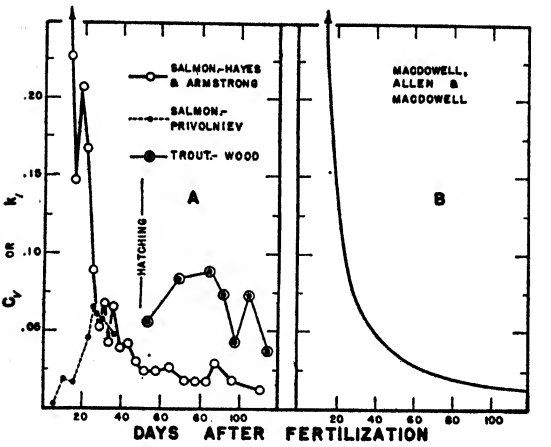


FIG. 6. *Relative growth rate of the salmon. A, main curve from Table I, Column 10; lower left from Privolniev's (19) salmon dry weights; right from Wood's (30) wet weights of trout reared at 12° C., with a time correction to make them fall above the corresponding salmon stages. B, smoothed by the method of MacDowell et al. (15), see Fig. 5.*

The important thing is not the magnitude of 1.47 which could be altered at will by changing the temperature, but the existence of a constant at all, suggesting as it does that the growth curve (Fig. 2) is of parabolic type.

The foregoing paragraphs are at variance with the conclusions to be drawn from Privolniev's (19) set of salmon dry weights. The chief difference lies in Privolniev's early C_v values, reproduced in Fig. 6A, showing the Minot curve rising at the start instead of falling. Privolniev's first values (for 35 embryos) were: three days, 17.3 mg.; nine days, 18.0 mg.; 17 days, 21.7 mg. Thus in the first three days the embryo must have grown nearly 17.3 mg., in the next six days only 0.7 mg. Moreover, it is stated that the formation of the embryo occurred at 11 days, or after the first two weighings, so that the latter must have included additional material. The rest of Privolniev's curve (not illustrated here) falls like the present one except for an interval after hatching when there is for a time, an apparent rise of doubtful validity. The rise shows much more clearly in Wood's (30) values, based on trout wet weights at 12°, which are also placed in Fig. 6A. The C_v values are from Wood's original data, but in placing the points on the figure, a time correction has been applied in order to make them fall above the corresponding developmental stages in the salmon.

For the beginning of the curve there are only Privolniev's values and the present ones, but for the interval after hatching there are several. Table II gives an opinion on a survey of these (not necessarily that of the author). The question is whether there is a hump on the Minot curve after hatching (and therefore whether the fish embryo differs from warm-blooded embryos in its habit of growth). The majority answer is certainly negative. If there is, at higher temperatures, as Wood's results suggest, an increase in the

TABLE II

ANSWERS TO THE QUESTION OF WHETHER THE RELATIVE GROWTH RATE, C_v, INCREASES AFTER HATCHING

Observer	Material	Wet wt. or dry wt.	Increased C_v after hatching
Kronfeld and Scheminzky (13)	Trout	Wet	No
Kronfeld and Scheminzky (13)	Trout	Dry	No
Gray (8)	Trout	Wet	No
Wood (30) at 12° C.	Trout	Wet	Yes
Wood (30) at 7° C.	Trout	Wet	Doubtful
Wood (30) at 3° C.	Trout	Wet	No
Privolniev (19)	Salmon	Dry	Doubtful
This work	Salmon	Wet	No

relative growth rate after hatching, it follows that hatching time must correspond to a minimum value. This would fit in with the finding of Hayes and Ross (11, p. 364) who showed that the storage of fat by salmon embryos is interrupted at the time of hatching. Obviously the effects of temperature on the shape of the growth rate curve deserve further investigation.

Fig. 6B shows the RGR as plotted by the method of MacDowell *et al.*, which Schmalhausen himself has come to use in his later papers (25). Fig. 7A gives the RGR according to Brody. The discontinuous nature of the curve

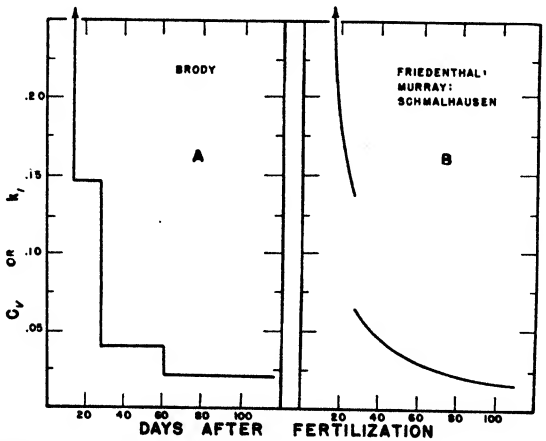


FIG. 7. *Relative growth rate of the salmon. A, calculated from a curve smoothed by the method of Brody (4), see Fig. 4. B, smoothed by the method of Friedenthal-Murray-Schmalhausen, see Fig. 5.*

is apparent and emphasizes again the individuality of Brody's views. Fig. 7B follows the method of Friedenthal-Murray-Schmalhausen, with a break in the centre corresponding to the central break in Brody's curve.

Since the RGR provides a most instructive approach to growth problems it is unfortunate that no unanimity of opinion exists as to the best means of representation. Statistical methods cannot provide the basis for a decision between the possibilities shown in Figs. 6 and 7, and in their absence the following conclusions are suggested:

1. When based on measurements of weight, a single general description of the whole curve is more to be desired than one which breaks it into parts.
2. Hence the modification of MacDowell *et al.* is to be preferred to the original method of Friedenthal-Murray-Schmalhausen.
3. While there may well be variations from time to time in the smooth descent of the RGR, the method of Brody does not provide a conclusive demonstration of them.
4. In view of the errors involved, measurements of weight would appear to lack sufficient sensitivity to serve for a detailed description of the RGR.
5. Progress in detailed description of the RGR is likely to come through a study of the fluctuations in embryonic respiration (20), the varying effect of temperature on differentiation and growth (14, 27, 28), and the times when special reactions such as glycogen synthesis are initiated by the embryo (10).

Growth in Length

Lengths of trout embryos have been measured by Willer (29) under hatchery conditions, with feeding begun at the usual time, and Allen (1) has published a series of such measurements on the salmon, which was not fed. Both find a complete growth cycle, like Fig. 1A, with the inflection, in the growth curve of the salmon, only 10 days after hatching. When length is plotted against time in Allen's curve the portion before the inflection is a straight line so that

$$l = a + bt.$$

Nevertheless the MacDowell formula will fit, hence

$$\frac{d \log l}{d \log (t - 9)} = 1.$$

With an abrupt change in slope the MacDowell equation also fits approximately the postinflection part of the curve, except for the final points, where growth had practically stopped. The inflection occurs long before the already discussed starvation inflection in weight, which some workers have described (8). Several conclusions may be drawn.

1. There can be no simple mathematical relation between length and weight such as

$$w = l^n$$

as has been suggested by Schmalhausen (23) for a variety of embryos.

2. Formulae such as that put forward by Backman (2) purporting to describe either weight or length curves with equal ease, cannot be used for a description of both processes in the same period of salmonid development. (Backman also uses area as an alternative unit).
3. Growth cycles in the sense of Sachs may be less general than is commonly thought, since a demonstration of their existence in terms of one unit does not constitute evidence about some other unit.

Acknowledgments

The authors are indebted to Prof. Charles Walmsley and Professor Donald Mainland for many helpful suggestions.

References

1. ALLEN, C. R. K. Proc. Nova Scotian Inst. Sci. 18 : 34-49. 1931-32.
2. BACKMAN, G. Ergeb. Physiol. 33 : 883-973. 1931.
3. BACKMAN, G. Skand. Arch. Physiol. 63 : 127-170. 1932.
4. BRODY, S. Univ. Missouri Coll. Agr. Research Bull. 97. 1927.
5. COURTIS, S. A. Growth, 1 : 155-174. 1937.
6. FRIEDENTHAL, H. Allgemeine und spezielle Physiologie des Menschenwachstums. Julius Springer, Berlin. 1914.
7. GLASER, O. Biol. Rev. Cambridge Phil. Soc. 13 (1) : 20-58. 1938.
8. GRAY, J. Brit. J. Exptl. Biol. 6 (2) : 110-124. 1928.
9. HAYES, F. R. and ARMSTRONG, F. H. Can. J. Research, D, 20 (5) : 99-114. 1942.
10. HAYES, F. R. and HOLLETT, A. Can. J. Research, D, 18 (2) : 53-65. 1940.
11. HAYES, F. R. and ROSS, D. M. Proc. Roy. Soc. (London) B, 121 : 358-375. 1936.
12. HOESSLIN, H. VON. Z. Biol. 90 (6) : 600-614. 1930.
13. KRONFELD, P. and SCHEMINZKY, F. Arch. Entwicklungsmech. Organ. 107 (1) : 129-153. 1926.
14. LJUBITZKY, A. I. Zool. Jahrb. Abt. Allgem. Zool. Physiol. 54 (4) : 405-422. 1935.
15. MACDOWELL, E. C., ALLEN, E., and MACDOWELL, C. G. J. Gen. Physiol. 11 (1) : 57-70. 1927.
16. MINOT, C. S. J. Physiol. 12 : 97-153. 1891.
17. MURRAY, H. A., JR. J. Gen. Physiol. 9 (1) : 39-48. 1925.
18. NEEDHAM, J. Chemical embryology. Cambridge University Press. 1931.
19. PRIVOLNIEV, T. I. Compt. rend. acad. sci. U.R.S.S. 3 : 419-421. 1935.
20. PRIVOLNIEV, T. I. Russ. Ark. Anat. Gistol. Embriol. 18 : Russian text and figures, 165-177. German text, 255-262. 1938.
21. ROBERTSON, T. B. The chemical basis of growth and senescence. J. B. Lippincott, Philadelphia. 1923.
22. SACHS, J. Arb. Botan. Inst. Würzburg, 1 : 99-192. 1874.
23. SCHMALHAUSEN, I. Arch. Entwicklungsmech. Organ. 109 (4) : 455-512. 1927.
24. SCHMALHAUSEN, I. Arch. Entwicklungsmech. Organ. 110 (1) : 33-62. 1927.
25. SCHMALHAUSEN, I. Arch. Entwicklungsmech. Organ. 124 (1) : 82-92. 1931.
26. THOMPSON, D'ARCY W. On growth and form. Cambridge University Press and The Macmillan Company. 1942.
27. TRIFONOVA, A. N., VERNIDOUBE, M. F., and PHILIPPOV, N. D. Acta Zool. Stockholm, 20 : 239-267. 1939.
28. VERNIDUB (VERNIDOUBE), M. F. Russ. Ark. Anat. Gistol. Embriol. 25 : Russian text, 68-79. English summary, 124. 1940.
29. WILLER, A. Atti Congr. Internat. Limnol. Teor. Applicata Roma (Verhandl. Internat. Verein Theoret. angew. Limnol.) 4 : 668-684. 1929.
30. WOOD, A. H. J. Exptl. Biol. 9 (3) : 271-276. 1932.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. MARCH, 1943 NUMBER 3

THE STANDARDIZING OF A LABORATORY METHOD FOR COMPARING THE TOXICITY OF CONTACT INSECTICIDES[1]

BY FRANK O. MORRISON[2]

Abstract

Toxicity tests were conducted with nicotine sulphate and nicotine alkaloid using *Drosophila melanogaster* as the test animal, with a modified Tattersfield atomizer spray machine, and by an immersion technique. One hundred and fifty flies were treated at each concentration each day. Each experiment was replicated 8 or 10 times using 3 to 22 concentrations. Data were analysed by the method of analysis of variance and by means of probits.

It appears from the data secured that careful standardization of any technique will be needed to secure comparable results. Results from spraying were the more uniform and consistent. Saponin spreader had a synergistic action with nicotine sulphate. It complicates results and its effect cannot be separated from that of the insecticide. Variations in observed mortalities result from different rates of spray application (slower applications were better), different ages of test animals (day-old flies and flies over four days old were most susceptible), different numbers of test animals per container (increased numbers increased the kill), different populations (these vary greatly in susceptibility), differences in larval and adult nutrition, and the use of different sized fly containers. All these factors must be standardized or accounted for. When this was done variations due to different experimenters were not significant.

In general six or eight replications were enough to establish a curve. Analysis of variance on angular transformation values gives a good test for consistency and the method of probits reveals much heterogeneity in the data.

Introduction

Tattersfield (36) has briefly but effectively reviewed the history of biological methods of testing insecticides. There is no need to repeat it here. Since that review O'Kane (27) has published the design of a new toximeter which has as its important characteristic the ability to spray the liquid on the insect from two directions, but greatly limits the number of test animals that may be used, and there has appeared a very excellent paper by Potter (32) emphasizing, as the following work does, the need of detailed standardization. The greater uniformity of results secured by Potter is at least in part due to the fact that all the data for one test were secured on one population of test animal. Both spraying and dipping methods have been used at Macdonald College for a number of years and a large accumulation of data has resulted. It is proposed to present much of these accumulated data in brief graphical and tabular form and to discuss them in the light of other published work.

[1] *Manuscript received in original form July 8, 1942, and as revised, December 4, 1942. Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Macdonald College Journal Series No. 175.*

[2] *Lecturer.*

Technique Used

Rearing and Handling of the Test Animal, Drosophila melanogaster

Bananas, partly peeled, dusted with dried brewer's yeast and placed horizontally on top of sawdust in candy-jars, and potato–yeast medium similarly used (34) proved the most successful rearing media. Other media (25) were not satisfactory for rearing large numbers of flies. Moreover, with the banana medium it was possible to add new food every few days and keep the nutritional condition of test animals reasonably uniform. Preliminary tests showed flies from exhausted media to be very susceptible to treatment. This effect of nutrition on susceptibility has been previously noted (24 and 31).

It was found convenient to cut out a portion of the tin tops of the candy-jars (Fig. 13) and replace it with fine copper wire gauze. Cultures were kept in chambers at 70° F. and approximately 80% humidity. New cultures were started by the addition of at least 500 flies to a newly prepared culture bottle. The population was allowed to build up (bananas being added as needed) for two weeks, but flies were not utilized for tests more than one month after the culture was started.

A candy-jar, the bottom of which had been replaced by copper wire gauze, was used as a collecting jar when it was desired to secure flies for tests. The top of a culture jar was unscrewed and a cover of two flat pieces of glass placed on it. Over these the collecting jar was inverted, the glass plates were withdrawn, and the culture jar and the inverted collecting jar were placed under a light, which induced the flies to enter the top jar. By manipulation of the two glass plates the collecting jar and the flies it contained could be removed and the culture left covered.

The flies were then blown, rather than shaken, into a glass separatory funnel through a cone-like adapter which fitted over the mouth of the collecting jar. When more than one culture was utilized for one experiment, all the flies were first blown into a reservoir jar and withdrawn as needed. This assured uniform sampling.

The separatory funnel could be held at a slight angle beneath an electric lamp and the flies crawled, when the large bore stopcock was open, into the outlet tube. This tube had been cut off about one inch beyond the stopcock and over it was placed a rubber collar which fitted snugly into the end of the small tubular fly containers. Flies might be easily counted as they passed the stopcock which acted as a gate to regulate the numbers passing.

The tubular fly containers were cut from glass tubing and were 45 mm. long by 14 mm. in diameter, inside measurement (Fig. 13). The flies were kept in the tubing by pieces of tulle, 28 mesh to the inch, stretched over the ends and held on by rubber bands. It was found advisable to preshrink new tulle for 24 hr. in 10% potassium hydroxide solution. One end of each container was covered prior to filling and the second end covered on removal from the collar of the separatory funnel.

When 150 flies were sprayed at one time in one container the container used was 22 mm. in diameter and 2½ in. long and the homeopathic vial into which treated flies were transferred was correspondingly large. Otherwise the handling was identical. When large numbers of flies had been immersed in one container they were transferred to filter-paper–lined Petri dishes rather than to homeopathic vials.

In all experiments except those specifically designed to test flies of different ages, flies used were from a few hours to four days old, the cultures being cleared of flies every four days. When flies of known age were required cultures were cleared each day and the flies retained in extra jars with a plentiful available food supply until required.

After being sprayed, specimens remained in the fly containers (which were moist with spray on all sides and through on the lower cover) for 30 min. during which time they were stored at 70° F. The flies were then removed by shaking or with a camel hair brush and placed in a homeopathic vial of the same diameter as the container. The vial was plugged with absorbent cotton previously dipped in a 5% honey solution and partly wrung out. Plugs were dipped just before use because, if dipped the day before, fermentation set in and the flies were stupified by gas accumulation in the vials. Mortality counts were made after 24 hr. storage at 70° F. All flies capable of movement were recorded as alive.

Spraying Technique

Spraying was carried out with a Tattersfield improved type atomizer (35) operated by a compressed air line (Fig. 13). A one-quarter h.p. motor supplied power to operate a small air pump, the air stream being directed through a reducing valve and filter to the atomizer and the pressure being measured by an attached manometer. An adjustable stand, glass fume cabinet, and clamp in which the containers could be supported directly beneath the cone of spray at a distance of 27 to 30 cm., as shown in Fig. 13, completed the apparatus. Spraying was carried out at a pressure of 15 lb. per square inch and the atomizer regulated to deliver 1 cc. of liquid in 20 sec.

Immersion Technique

Shell vials of the same diameter as the tubular fly cages used in spraying tests were utilized. Flies were counted into these and imprisoned by a loose fitting plug of non-absorbent cotton pushed down to near the bottom. The material to be tested was introduced by means of a drawn out glass tube with a syringe bulb at one end. It was withdrawn the same way. The fluid was allowed to rise above the plug, thus all specimens were submerged. All specimens were kept immersed for 30 min., a period that Gilbert and Marshall (19) found equivalent to spraying with 1 cc. and subsequent storage for 24 hr.

Morley (26) carried out immersions by submerging the flies in the regular spray-type of fly container, which was immersed in a large beaker of solution. Submersion was maintained for 20 min. instead of 30.

Making up Dilutions of Nicotine

The nicotine used was in the form of nicotine sulphate, as put out by Canadian Industries Limited, in commercial pound tins and labelled 40%, and in the form of the alkaloid put up by Kentucky Byproducts Co. Ltd., under the trade name of Nicofume and labelled 40%. On opening a new tin the concentration of nicotine was tested by the standard A.O.A.C. method of analysis (precipitation with silicotungstic acid, etc.). This analysis was repeated at intervals over a period of time. Attempts of the earlier workers to make up 20% stock solutions were given up as precipitates formed which clogged the atomizer. Each day a 2 or 4% stock solution was prepared from the tin, using the determination figures secured in the analysis, and the desired concentrations made up from this. Concentration throughout is expressed uniformly in weight (gm.) of the alkaloid nicotine present per 100 cc. of solution.

Design of the Experiments

In general the scheme adopted was to test the flies in lots of 15. Ten fly containers, each of 15 flies, were tested at each concentration. Where possible sufficient concentrations were used to give a picture of the complete range of toxicity. All concentrations were tested on the same day (same population) and the experiment replicated on 10 different days. Ten containers were treated with distilled water as a check. Modifications of this plan were necessary on one or two occasions. These are indicated in the following section. The object was to secure results on statistically significant numbers.

The following are the materials tested, and other phases of the subject investigated.

1. *By the Spray Technique*
   - *a.* Nicotine sulphate (11).
   - *b.* Nicotine sulphate in a 1% saponin solution.
   - *c.* Nicotine alkaloid ("Nicofume").
   - *d.* Nicotine alkaloid ("Nicofume") in a 1% saponin solution.
   - *e.* Nicotine sulphate in 1% saponin solution on flies of different known ages.
   - *f.* Nicotine sulphate sprayed at different rates.
   - *g.* Nicotine sulphate sprayed on 10 containers of 15 flies each and on one container of 150 flies.
   - *h.* Nicotine sulphate, saponin, and nicotine sulphate plus saponin tested on samples of the same population to determine if the saponin in combination acts synergistically.
   - *i.* Nicotine sulphate on fly samples reared on bananas and on potato–yeast medium.
   - *j.* Nicotine sulphate used on containers of 5, 10, 15, 30, 75, and 150 flies, respectively.
   - *k.* Nicotine sulphate tested by five experimenters on the same fly populations.
   - *l.* Nicotine sulphate tested on fly samples sprayed in containers of different diameters.

2. *By the Immersion Technique*
   a. Nicotine sulphate.
   b. Nicotine sulphate using 10 containers of 15 flies each and one container of 150 flies.
   c. Nicotine sulphate in a 1% saponin solution.
   d. Nicotine alkaloid ("Nicofume").
   e. Nicotine alkaloid ("Nicofume") in a 1% saponin solution.

Discussion

The Evaluation of Mortality Data

It was the purpose of this investigation to study the standardization of a technique for comparing contact insecticides. It was recognized that the test animal had no economic importance, and that the results could have no direct application to control work.

In order to ascertain the value of the technique some method of the appraisal of results is essential. Tattersfield (36) has summarized the literature on methods of assessing results. He suggests that toxic action may be judged in three ways: "(*a*) by the effect produced by different concentrations in a given time, (*b*) by the effect produced at different intervals of time, the concentrations being kept constant, (*c*) by the effects produced at different intervals of time by different concentrations." The first of these, (*a*), giving rise to dosage–mortality curves is our only concern here.

To test our technique we should have some method of testing the consistency of results from different replications and from repetitions of complete experiments.

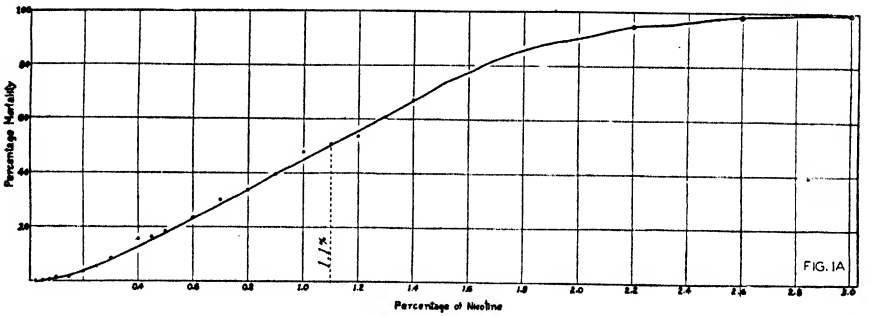
Cameron and Prebble (9) considered the per cent mortality secured from each container of 15 flies as a normal variate and worked out from these a standard deviation of the mean value of each mortality for each experiment. These formed a test of consistency, but were subject to errors arising from slightly different numbers in different containers from which the percentage mortalities (later averaged) were derived. It was suggested by these workers and by Dr. Hopkins of the National Research Council that if each concentration were tested each day, the percentage mortality for each replication (percentage derived from the totals dead and alive at each concentration, i.e., in 10 containers) might be treated as a normal variate, and the entire experiment subjected to analysis of variance. This set-up was adopted by the writer. However, on completing the work, and on further correspondence with Dr. Hopkins, it was concluded that the analysis of variance procedure should not be applied to data in which the percentage kill varies from 0 to 100, since the expected variance at the different dosages would not be the same (1, 6, 7, and 12). In order that the whole of the observations may be employed in the estimation of a common error, which will thus be computed with considerable accuracy, each variate can be transformed to a value in terms of angles of equal information (6, 7) and as this equalizes the expected variance

from each treatment, the analysis of variance procedure is justifiable. It was pointed out by Dr. Hopkins that the design of the experiment whereby each dosage was tested each day allowed for the elimination of the sum of squares between replicates from the experimental error and greatly reduced this value.

The average kill for each concentration in terms of angles and the standard error of the mean kill for any concentration is shown for each experiment in the tables given. The value of this method as a measure of the consistency of the data is evident. Where different numbers of replicates have been tested, it measures the evident greater consistency with increased numbers. Applied to immersion and spray results, it will be pointed out that the data from the spray technique show the greater consistency.

Reproducibility of Results

A logical question asked by people who must make routine tests of comparative toxicity is, "How many times must each test be replicated in order to secure reliable results?" All attempts to answer this question have proved futile because of the marked inconsistencies between the results of successive tests. In one test, cited below, an increase of two replications actually increased the error of the mean. Under these conditions our search must be



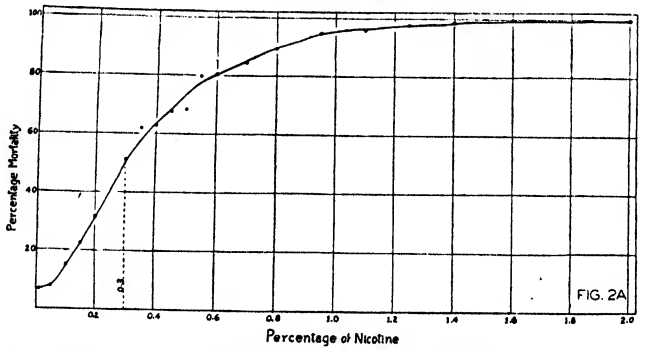


FIG. 1A. *Sigmoid–dosage–mortality curve of nicotine sulphate used as a spray (11).*
FIG. 2A. *Sigmoid–dosage–mortality curve of nicotine sulphate in a 1% saponin solution used as a spray.*

first for means of getting consistent results rather than for the optimum number of replicates.

Table I*d*, and Figs. 2A, 2B*a*, and 2B*b* are an attempt to throw light on this discussion. The first four replications, then the first six and the first eight have been compared with the 10 replications for one representative experiment. Any of the other experiments would, no doubt, show similar trends. If one merely examines the results and the sigmoid curve, it appears that increasing the replications gives progressively better results, i.e., by the time 10 replicates have been averaged each successively larger dosage gives rise to a higher mortality, and hence allows for a better fitting curve. This is as we would expect—the larger the number of populations sampled the more closely the averages fit a definite curve.

Each group of replicates was subjected to an analysis of variance and the resulting variance, expressed as angles of equal information, indicates the least variation of the data from the experiment mean when six replicates were used, the standard deviations of experiments of 4, 6, 8, and 10 replicates being 3.28°, 2.51°, 3.43°, and 4.59° respectively. When these are divided by the root of the number of variates in a mean to secure the standard error of the mean the order remains the same though 8 and 10 replications are then seen to have an advantage over four. The standard errors of the means work out to be 1.64°, 1.02°, 1.21°, and 1.45° respectively.

It is especially significant that in each analysis of variance the variation between replicates is significantly three or four times as large as the residual error. Similar figures for variance due to replications occurred in all the other experiments, as is shown by the following table.

Experiment	Variance between replicates	Residual error
Nicotine sulphate + saponin spray (4 repl.)	104.53	10.82
Nicotine sulphate + saponin spray (6 repl.)	277.05	6.29
Nicotine sulphate + saponin spray (8 repl.)	274.57	11.73
Nicotine sulphate + saponin spray (10 repl.)	335.48	21.09
Nicofume spray	137.15	15.72
Nicofume spray + saponin spray	489.16	17.70
Nicotine sulphate immersion	389.23	47.47
Nicotine sulphate + saponin immersion	267.63	19.08
Nicofume immersion	224.25	35.20
Nicofume + saponin immersion	90.97	24.78
Nicotine sulphate + saponin spray		
1 cc. per 10 sec.	267.35	27.18
1 cc. per 25 sec.	222.26	20.72
Nicotine sulphate spray		
15 flies per container	144.81	26.22
150 flies per container	141.78	49.59
Comparison of nicotine sulphate and nicotine sulphate + saponin	270.37	25.24
Comparison of different container diameters	134.27	43.46
Comparison of different workers	171.70	44.29

This indicates significant variation of the susceptibility of populations. It makes the completion of one replicate each day essential as the lower range of a curve calculated on today's population is not comparable with the higher range calculated tomorrow. Accurate comparative work involving the comparison of a material under investigation with one better known may have to be done on the same populations. Thus instead of a previously determined standard curve or one based on cumulative data, as suggested by Bliss, the standard material may have to be tested each time along with the new material on the same populations, as is done now in some biological assay work.

The variances in the above table are measured in degrees. Dr. Hopkins has pointed out that there is certain variation inherent in the value θ. This theoretical expected remainder, due to chance alone, is represented by the formula $V\theta = \frac{8100}{\pi^2 n}$. Assuming n in all tests conducted to be 150 (the number of flies tested per replication), $V\theta = 4.57$. If we examine the table, we will observe that the residual error variance varies from 6.29 to 49.59. In all tests except one it is over twice the anticipated value due to chance alone and in 13 out of 17 experiments it is well over three times this value. The technique can evidently be still further refined to eliminate the sources of this variance. What they may be we can only guess but slight variations in temperature during the experiment and unobservable differences in the methods of transferring flies from containers to vials, may account for some of it as may also varying sex ratios in different containers. Potter (32) presents evidence that indicates that at least some of this variation is due to "unequal administration of the dose" and he suggests that the microclimate around individual insects between spraying and counting may be an important factor.

Mortality–Dosage Relationship

It was early pointed out (40) that since the sigmoid–dosage–mortality curve tails off at its upper and lower ends, the best comparison of two insecticides is possible near the 50% kill point or so-called Median Lethal Dose or *L.D.* 50. This point is also discussed by Bliss (5). Clark (12) and others have discussed the interpretation of the sigmoid curve, and Bliss (3), O'Kane (28), Gaddum (18), Hemmingsen (21), and Bliss (4, 7, 8) have suggested conversion values that would reduce the curve to a straight line and make comparison based on the entire line (complete range of kill) possible. The tables of Bliss have been widely used and Cochran, in a supplement to Tattersfield's and Martin's paper (38), developed a numerical comparison of the toxicity of two insecticides.

In view of the wide acceptance of this method, a probit–log–dosage regression line has been fitted to each set of data secured by the writer. The methods including the weighting schemes given by Bliss (4) have been used. The variance of the position (Va) and the variance of the slope (Vb) are in-

dicated. In view of the much larger number of points determined and specimens used than in previously published data, the calculations involved proved an arduous task. If each mean for each replication were taken as a separate variate and thus the number of degrees of freedom for Chi^2 greatly increased the labour involved would also be increased, though such a method should be more accurate, as evidently populations vary considerably from day to day.

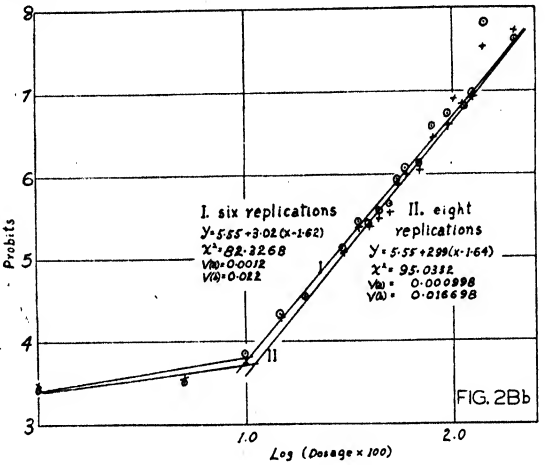
The consistency of the data from successive replications has been tested by this method in one experiment (Table I*c*). Using Chi^2 as the test of the goodness of fit, the results from the first four replicates conform more closely with the theory than do those of any larger number. When the first four and following four replications of the tests with nicotine sulphate and saponin used as a spray are considered as separate experiments and interpreted by the method of probits (5), the formulae 18, 19, and 20 (p. 316) of Bliss's article may be used to test the consistency of the two sets of data. When this is done (Figs. 2B*a*, 2B*b*, and 12), the Chi^2 for difference in position equals 208.376 and the Chi^2 for difference in slope equals 0.2203. It is thus seen that the lines have almost the same slope but differ widely in position and, in the words of Bliss, the new data "are not sufficiently consistent with the original experiments to warrant including them in a common dosage mortality curve." But these data are part of the same experiment during the progress of which there seems to have been a definite and steady decrease in susceptibility over the whole range and hence a shift of the regression line downward. It seems probable that the data from any of the other experiments would show similar inconsistencies.

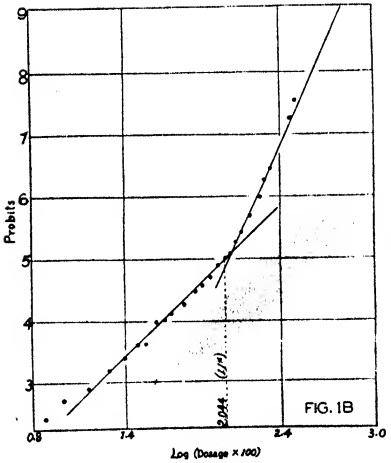
When the regression line for each replicate in this set of data is calculated separately (Table I*c*), it will be observed that the slopes of the lines are reasonably parallel except for that of Replicate 10. Where the slopes of the lines of two materials differ widely, comparison of toxicities (or susceptibilities) may have to be made at some point other than the *L.D.* 50.

If we consider the data in Table IX and Fig. 10A where the same technique and material were employed but the rates of application were 1 cc. per 10 sec. and 1 per 25 sec. as compared to the rate used throughout other experiments (1 cc. per 20 sec.) the very differences in the *L.D.* 50's would indicate that these data are not comparable. Less easily justified inconsistencies or evidences of unexplainable variation are shown by the *L.D.* 50 of 1.1% secured by Cannon for nicotine sulphate used as a spray and the *L.D.* 50 of 0.89% secured by the writer with similar material and technique when the numbers of flies per cage were being tested, as well as by the inconceivable differences in the results with nicotine sulphate and saponin spray when tested first with different numbers of replications and later on flies of different ages.

In the face of this evidence it becomes difficult to say what constitutes the best number of replications. If these unexplainable variations are due to

seasonal effect on susceptibility or any other long term effect, the fact that would matter would not be the number of replications alone but the number related to the period of time required to conduct them. That the cause is a "long term" one is suggested by the much greater consistency of data from successive replications than of data from experiments conducted at widely different times. Potter (32) was unable to account for changes in the resistance of the population as a whole, in the course of a few generations.





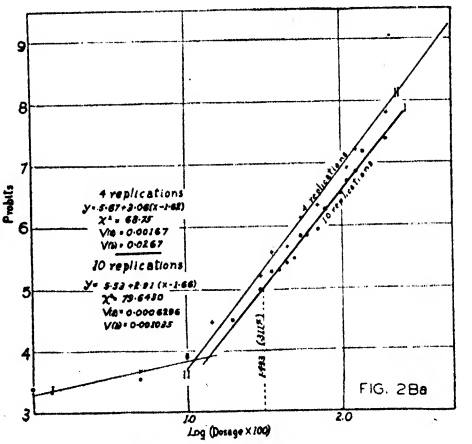
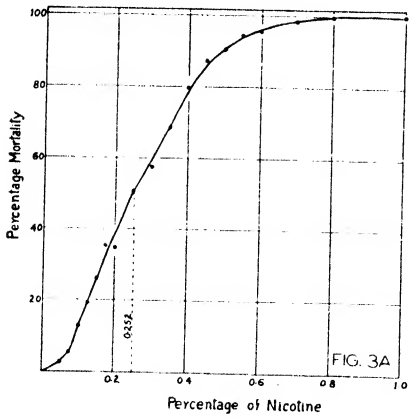


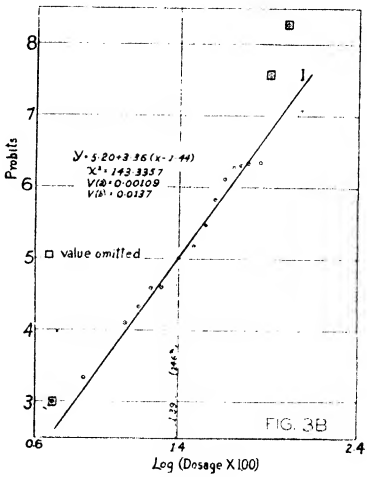
FIG. 1B. *Probit–log–dosage regression line of nicotine sulphate used as a spray fitted by eye to the data of Cannon (11).*

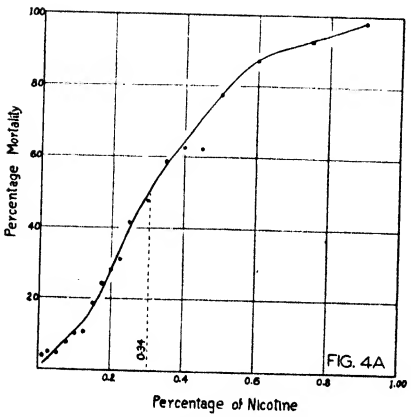
FIG. 2Ba. *Probit–log–dosage regression lines for nicotine sulphate in a 1% saponin solution used as a spray, when 4 and 10 replications are considered. (The lines were fitted by the method of Bliss (5) and the corresponding Chi² and variances are given.)*

FIG. 2Bb. *Probit–log–dosage regression lines for nicotine sulphate in a 1% saponin solution used as a spray, when six and eight replications are considered. In the expression,* $y = 5.55 + 299\ (x - 1.64)$, *read 2.99 for 299.*

Accepting Bliss's criterion for homogeneity, i.e., a Chi² not greater than the one for which the corresponding P in Fisher's Table III is 0.05, only the test involving nicotine sulphate and saponin sprayed on two-day-old flies shows this quality in the test animals. In that test only three points on the curve were determined. Heterogeneity of the data is thus generally indicated in all cases. In many experiments the very large Chi² suggests that the theory involved in the application of the method of probits probably does not describe the data and some curve other than a straight line would fit the converted data better. In fact the existence of variations from the regression lines is established. It is the nature of these variations that should be investi-







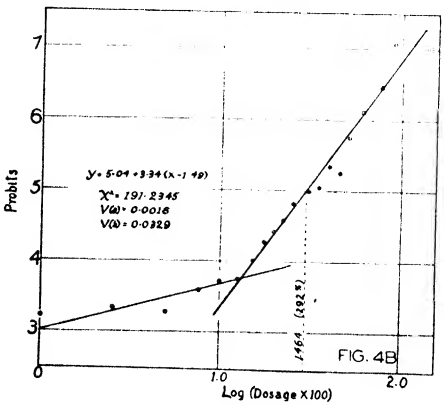


FIG. 3A. *Sigmoid–dosage–mortality curve for "nicofume" spray.*
FIG. 3B. *Probit–log–dosage regression line for "nicofume" spray.*
FIG. 4A. *Sigmoid–dosage–mortality curve for "nicofume" in 1% saponin used as a spray.*
FIG. 4B. *Probit–log–dosage regression line for "nicofume" in 1% saponin used as a spray. In the expression, $y = 5.04 + 3.34\ (x - 149)$, read 1.49 for 149.*

gated. Thus the underlying relationship may be linear and these variations random in their nature and simply due to faulty manipulations and heterogeneous experimental material along with pure sampling errors, or the mortality–log–dosage relationship may not be truly linear so that a better fitting line would be obtained by including quadratic or higher order terms. It is the hope of the writer that these or similar data may, in the future, be worked over with this matter in mind.

Factors Specifically Tested

Several specific factors significantly affect the results secured in these tests. Nevertheless standardization of these factors alone does not seem to assure results reproducible within a desirably small experimental error. All experiments conducted exhibit a residual error sufficiently in excess of the expected random sampling fluctuations to suggest that in no test was a state of statistical control secured. In fact a statistical reviewer has pointed out to the writer that the title "standardizing of a laboratory method" is in truth a misnomer as standardization has not been effected. Further work is essential to refine experimental methods in such a way as to insure reproducible results within a predictable error. However, the extensive tests reported here throw much light on the difficulties involved in this type of test work.

The data secured lend themselves to the illustration of the following points:

THE COMPARATIVE VALUE OF SPRAYING AND IMMERSION TECHNIQUES

Experiment	(1) Standard deviation for the experiment by analysis of variance (in angles of equal information)	(2) Chi^2 derived from comparison of a fitted probit–log–dosage regression line with the data
	Immersion technique	
Using nicotine sulphate	6.90°	134
Using nicotine sulphate and saponin	4.39°	95
Using nicotine and saponin	5.02°	111
Using nicotine	5.88°	309
	Spray technique	
Using nicotine sulphate and saponin	4.59°	60
Using nicotine and saponin	4.21°	191
Using nicotine	3.95°	143

From the above table, Column 1, it is evident that data secured by the spraying technique were more consistent than those secured by immersion. Column 2, however, based on the probit scheme, fails to indicate any greater conformity of the data to the theory. The greater inconsistency in results from immersion probably arises from the difficulty experienced in getting the fluid uniformly in contact with the flies. A container of immersed flies, when held up to the light, always showed varying amounts of air held as

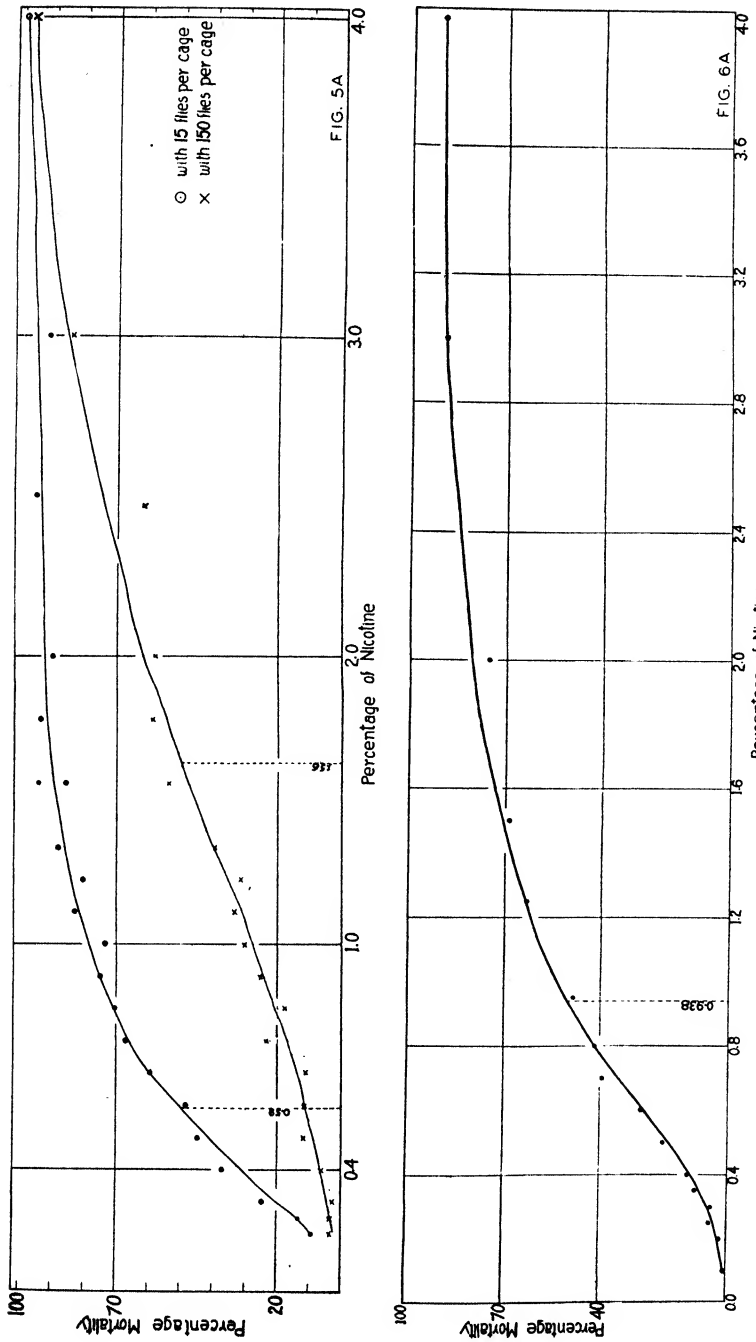


FIG. 5A. *Sigmoid–dosage–mortality curves for nicotine sulphate by the immersion technique.*
FIG. 6A. *Sigmoid–dosage–mortality curve obtained using nicotine sulphate in 1% saponin by the immersion technique.*

small bubbles by the setae of the flies. This might be overcome with different test animals (14). The retention of bubbles was especially evident when saponin was used. The spreader reduced the surface tension of the solution. It is especially noticeable that though it greatly enhanced the killing power of nicotine sulphate used as a spray, it did not do so to any extent when immersion was practised and with the latter technique 100% kill was very difficult to attain. It is possible that the reduced surface tension of the mixture did not allow sufficient of a film for the slow formation of gaseous nicotine from nicotine sulphate, to build up lethal concentrations before the film dried up.

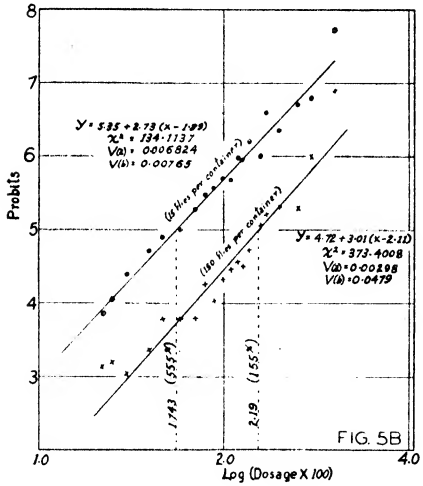
Table IV*b* has been included to illustrate how slight variations in technique between two workers may entirely vitiate results. If any one row of the results in this table is examined, a regular alternation of high and low values for successive concentrations may be observed, while the row above or below shows a similar alternation with concentrations that previously gave high kills now showing low ones. In order to complete all concentrations in one day, the writer and his assistant found it necessary for each to carry out treatments at the same time. To obviate any possible effect of this arrangement, it was decided that the concentrations tested by the writer one day would be tested by his assistant the next day. The techniques used came, in time, to differ slightly. After the immersion was complete, the fluid was removed with a glass tube and syringe bulb. The cotton plug was removed with forceps. Often many or all flies adhered to this plug. Excess fluid was drawn from the plug with the syringe. The flies were then transferred to the homeopathic vials. For this last process, the writer consistently used a comparatively dry brush with the excess water removed from it by pressing it on the edge of the beaker of distilled water (in which it was kept) each time it was removed. The mortalities thus secured were comparatively high. The assistant used a saturated brush the excess water from which was absorbed by the partially dry cotton plug. The mortalities secured were comparatively low. Presumably the water passing from the brush to the plug washed from the flies the adherent film of toxic substance. Correction of this variation resulted in uniform mortalities.

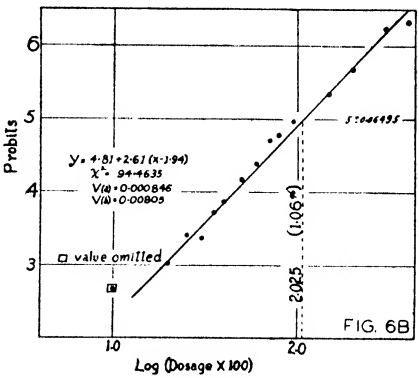
It is obvious that a test as sensitive as this would be difficult to standardize so that results might be duplicated by different workers.

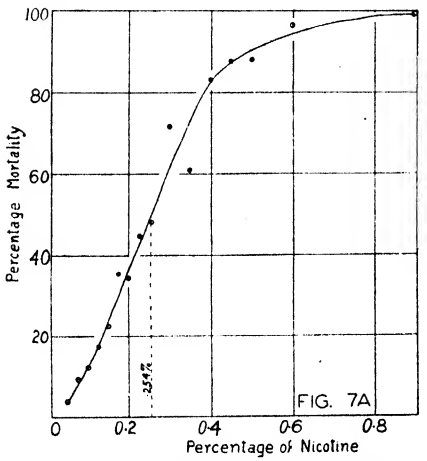
The Use of a Spreader

It is logical that introducing a third compound will complicate results. Saponin itself is toxic to *Drosophila*. In tests involving this spreader the toxicity of the saponin alone was tested. It gave an average mortality of 5%. Cameron and Prebble (9) corrected all their data on the basis of the "saponin check mortality" using the well known Abbott's formula. This correction assumes the spreader and nicotine to act separately. The present writer made no such corrections except for "water check mortalities", when these exceeded 3%. The Abbott correction, applied to those sets of data where the lower end of the probit graph fits poorly, would appear to improve this fit

(Figs. 5B and 7B). Saponin tested with nicotine sulphate by the spray technique increases the mortality approximately four times whereas used with "Nicofume" it raises the toxicity only slightly above that of "Nicofume" alone. On the other hand, nicotine sulphate alone proved more toxic than when used with saponin by the immersion technique.







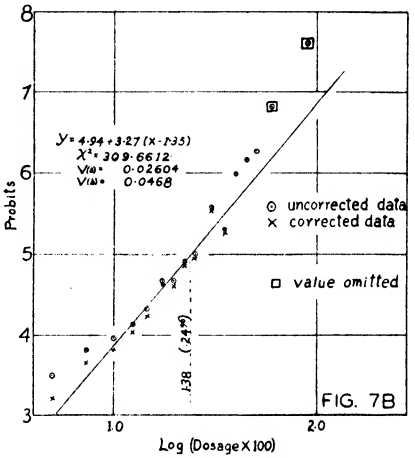


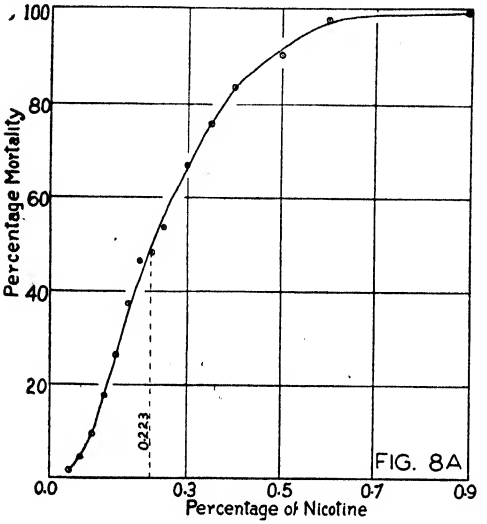
FIG. 5B. *Probit–log–dosage regression lines for nicotine sulphate by the immersion technique.*

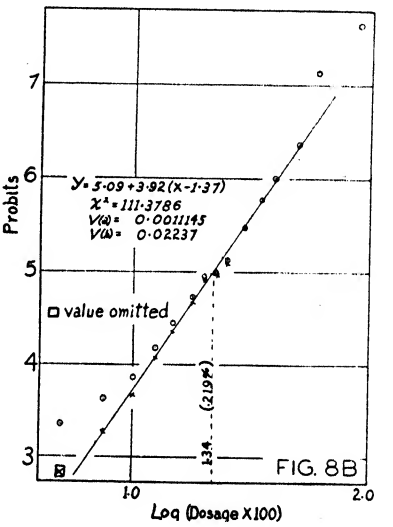
FIG. 6B. *Probit–log–dosage regression line for nicotine sulphate in 1% saponin tested by the immersion technique.*

FIG. 7A. *Sigmoid–dosage–mortality curve obtained using "nicofume" by the immersion technique.*

FIG. 7B. *Probit–log–dosage regression line for "nicofume" tested by the immersion technique.*

Bliss (8) has discussed the phenomenon of synergism and the effect of different types of joint action on the form of the fitted probit–log–dosage regression line. If we compare Fig. 1B and Figs. 2B*a* and 2B*b* by Bliss's standards, we are inclined to see definite evidence of synergism in Figs. 2B*a* and 2B*b*. However, this sweeping statement is hard to justify since Fig. 1B





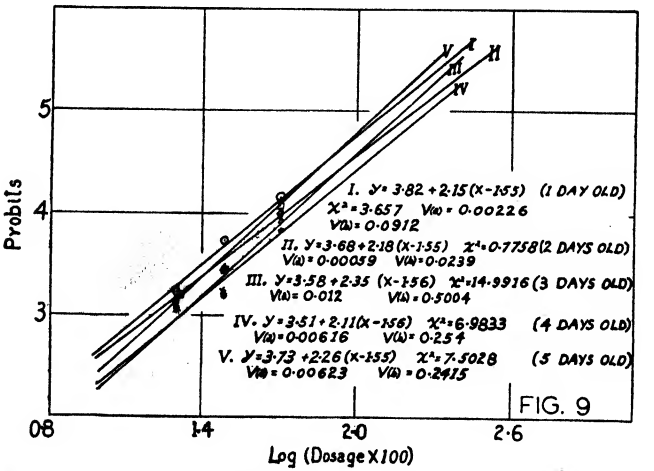


FIG. 8A. *Sigmoid–dosage–mortality curve obtained using "nicofume" in a 1% saponin solution by the immersion technique.*

FIG. 8B. *Probit–log–dosage regression line for "nicofume" in a 1% saponin solution by the immersion technique.*

FIG. 9. *Probit–log–dosage regression lines for nicotine sulphate in a 1% saponin solution tested as a spray on 1-, 2-, 3-, 4-, and 5-day-old flies.*

itself would appear to evidence the individual and separate action of two materials. Yet Fig. 10B compares favourably with Figs. 2B*a* and 2B*b* and gives evidence that results can be reproduced. The conclusion that there is a synergistic effect between the saponin and nicotine sulphate when used as a spray is borne out by the great increase in the toxicity when the mixture is used. This is in keeping with the work of Maxwell (24), who points out that various soap spreaders have a large adjuvant value with nicotine sulphate used as a spray but little when used with the alkaloid. When the immersion technique was used (Figs. 5B and 6B) the effect, though much less, must fall in either Bliss's Type 2 "similar joint action" or Type 3 "synergistic action".

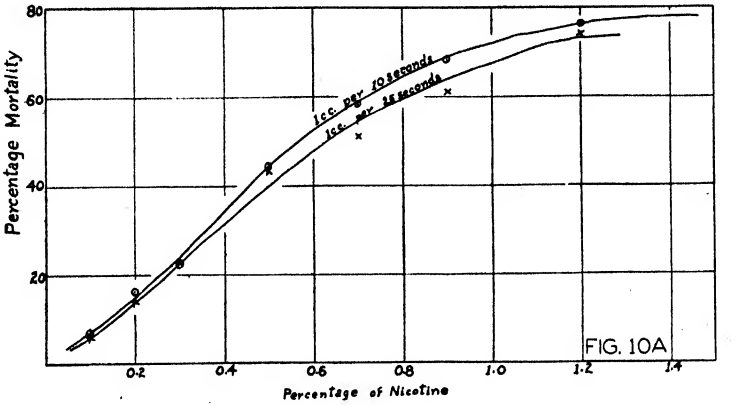
But now consider the "Nicofume" results. By the spray technique (Figs. 3B and 4B), it appears that the saponin may act independently, the upper part of the second curve being little changed, while by the immersion technique (Figs. 7B and 8B) no effect is evident.

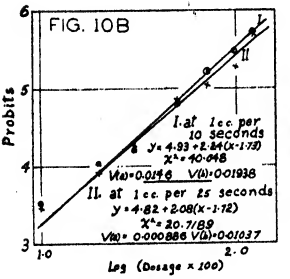
On the suggestion of Dr. Hopkins an experiment was set up to measure the synergistic effect of saponin used with nicotine sulphate. Eight replicates of 150 flies each (tested as before in containers of 15 flies each) were tested at concentrations of 0%, 1.1%, and 1.5% nicotine. Each concentration was used alone and in combination with 0%, 1%, and 3% saponin (Table XI). The resulting mortalities were transformed to Bliss's Angles of Equal Information, and set up as a 3 × 3 table, for analysis. The interaction between saponin and nicotine, if significant, should indicate a synergistic action. The following were the results:

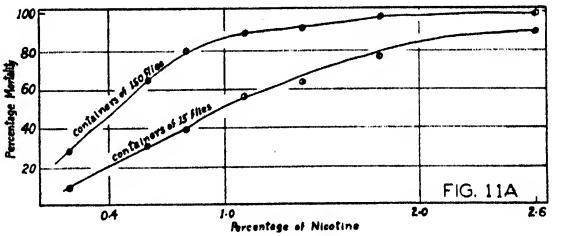
Source of variance	Sums of squares	Degrees of freedom	Variance	*F*	*F* for 5% *P*	*F* for 1% *P*
Variance for replicates	270.37	7	38.62	1.53	2.25	3.12
Variance for treatments						
(*a*) for conc. of nicotine	32561.47	2	16280.74	645.04	3.15	4.98
(*b*) for conc. of saponin	4218.41	2	2809.20	83.56	3.15	4.98
Variance for interaction (nicotine × saponin)	321.98	4	80.50	3.19	2.56	3.72
Error	1413.63	56	25.24			
Total	38785.86	71				

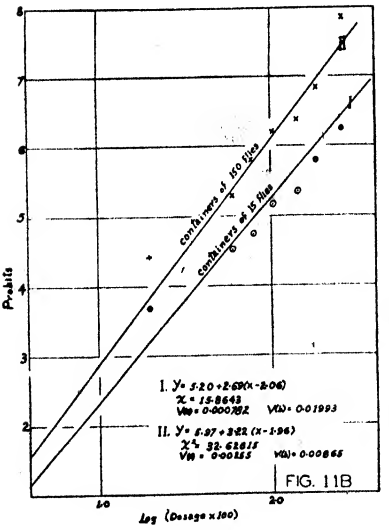
The interaction is seen to be within the range of significance. On examination of the data Dr. Hopkins has suggested that the interaction (hence degree of synergism) is much more pronounced than this analysis suggests. It is essentially the same for each concentration of the two tested. To show this a computation of the component of interaction variance ascribable to the single degree of freedom representing the difference in mortality between no nicotine and either 1.1 or 1.5% in the absence of saponin and in the presence of 1 or 3% saponin may be made.

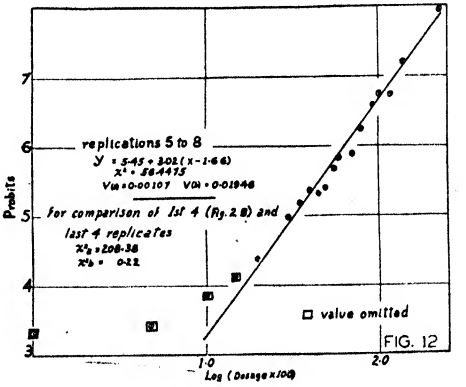
The interaction variance for this single degree of freedom accounts for no less than 277.14 of the total 321.98 and thus significantly exceeds the mean square error. It is thus established that in this experiment a measurably significant synergistic effect was apparent.











The addition of the saponin made regular and even spraying difficult as it interfered with the action of the atomizer. To explain the drop in mortality resulting from the same mixture as in Fig. 2B*a* (*L.D.* 50—0.311%), Fig. 9 (*L.D.* 50 from 0.12 to 0.22%), Fig. 10A (*L.D.* 50—0.46 to 0.63%), and Fig. 11A (*L.D.* 50—0.55 to 0.7%) preliminary tests were carried out on different samples of saponin varying from crude to chemically pure but no evidence of important variations in effect was obtained.

It is evident that we cannot gauge the nature or extent of the effect of a spreader on toxicity, and that it simplifies the comparison of insecticides when the spreader can be left out of consideration.

The Rate of Application

Figs. 10A and B indicate that the rate of application of the spray at least within the limits tested is not as important a factor, when animals as large and active as *Drosophila* are sprayed through tulle, as one might expect. The slower rate, however, gave the more consistent results from the point of view of the fit of a regression curve ($Chi^2 = 21$ for the slow rate as compared to 40 for the more rapid) and also gave data with less variance (S.d. 4.55° as compared to 5.21°).

The Age of the Test Animal

Cameron and Prebble (9) (Table VIII*c*) using flies not over three days old and flies not over four days old found considerable difference in susceptibility. These workers used nicotine sulphate plus saponin and secured kills comparable with those first obtained by the writer with these materials.

Maxwell (24) using flies of known ages found one-day–old flies much more susceptible than flies at other ages and three-day–old flies the most resistant forms tested.

Tables VIII*a* and VIII*b* and Fig. 9 indicate results secured in experiments by the writer. At a glance it is evident that the average kill given by what was previously the *L.D.* 50 was now less than 10%. No error could be found in the dilutions but it is expected one must have occurred. The nicotine sulphate when analysed gave the same percentage nicotine as before and used alone at 1.1% (*L.D.* 50 of Cannon's work) gave 58.6% kill. The laboratory temperature ranged 3° to 5° C. lower than during the earlier work. Any other variation would appear to be due to a new supply of saponin but varia-

---

FIG. 10A. *Sigmoid–dosage–mortality curves obtained testing nicotine sulphate in 1% saponin solution by the spray technique.*

FIG. 10B. *Probit–log–dosage regression lines for nicotine sulphate tested in 1% saponin solution by the spray technique. For $V(a) = 0.0146$, read $V(a) = 0.00146$.*

FIG. 11A. *Sigmoid–dosage–mortality curves obtained using nicotine sulphate spray.*

FIG. 11B. *Probit–log–dosage regression lines for nicotine sulphate tested as a spray. For $\chi = 15.8643$, read $\chi^2 = 15.8643$.*

FIG. 12. *Probit–log–dosage regression line for nicotine sulphate in a 1% saponin solution used as a spray. Replications 5 to 8 inclusive are considered. The Chi^2 values for the comparison of the position and slope of this line with that for the first four replications (Figs. 2Ba and 2Bb are included). For Fig. 2B, read Fig. 2Ba.*

tions in the quality of the crude saponin used were not demonstrated by preliminary tests.

The greatest differences between any two ages occurred between one-day–old flies treated with 0.3% nicotine (kill 10%) and five-day–old flies treated with the same dosage (kill 5.9%). If each of the 10-percentage kills for the 10 replications be treated as a normal variate an error of the mean for each treatment may be secured. These errors being determined, we find the error of the difference between the two means to be 2.24%. The difference cannot be called significant. Similarly the mortality of one-day–old flies treated with 0.5% nicotine was 21.8%, while that of four-day–old flies was only 11.5% but the error of the difference between these two means is 8.10%. However, if the errors be determined simply as errors of sampling, the formula $\alpha = \sqrt{\frac{PQ}{N}}$ being used, the errors of these differences are much smaller.

The probit–log–dosage regression lines for these results have been calculated and drawn (Fig. 9). The goodness of fit of these lines varies and cannot of course be considered as valuable a test where only three points are used, as this measure of fit must obviously depend on one point. Moreover, with the low kills obtained, the lines may not be the true ones. It will be recalled (5) that the data secured at low concentrations are frequently inconsistent with the straight line hypothesis.

A second experiment was carried out at a later date using nicotine sulphate alone as a spray, and one concentration only, the *L.D.* 50 of Cannon's work (11), 1.1% (Table VIII*b*).

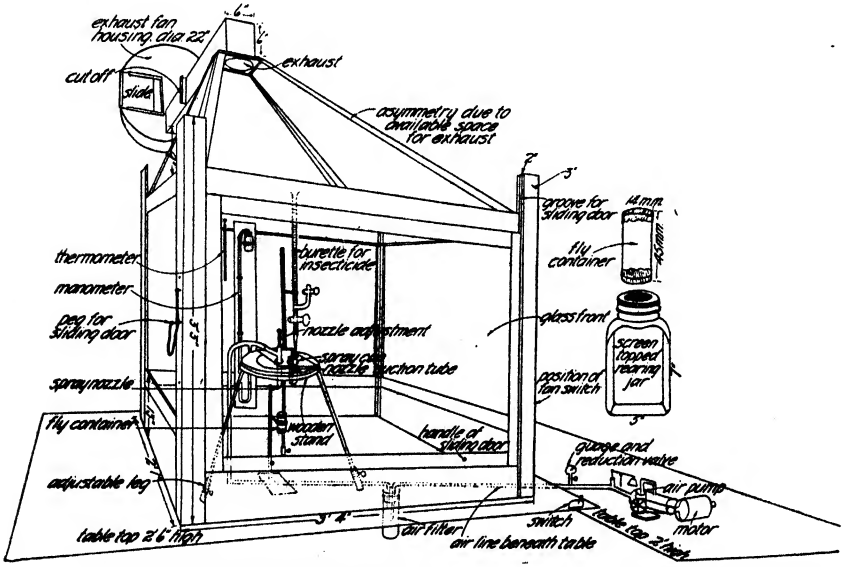


FIG. 13. *Glass cabinet housing modified Tattersfield atomizer, rearing jar, and fly container.*

It would appear that age affects susceptibility to some extent. Probably about 10% of the flies in each population used were older than the estimate owing to the impossibility of removing all the flies from a culture at one time. On two occasions results with certain populations showed unusually high susceptibility and investigation proved that the bananas supplied as food had hardened on the surface and were not available. These results were discarded, but the suggestion remains that nutrition is an important factor in governing susceptibility (24).

The Number of Test Animals per Container

It was the contention of Maxwell (24) that the treating of a larger number of flies in the same container would reduce the labour and the sampling error. The sampling error, however, is more dependent on the number of populations sampled than on whether the sample be treated all at once or in batches.

Tables IV*a* and IV*b*, Fig. 5A and Table X, Figs. 11A and B give results of experiments in which the treatment of 10 containers of 15 flies each has been compared with the treatment of 150 flies in one container. The same populations were sampled in each test. In so far as possible the techniques were similar for the containers of various sizes. Only 1 cc. of spray was used per container, regardless of the number of flies involved.

If we consider the results by immersion, the differences in the *L.D.* 50 and the dissimilarity in the curves suggest that variations in numbers per cage totally upset the results. If we accept the probit–log–dosage regression line interpretation the measure of goodness of fit shows the smaller numbers to be more satisfactory. When the spray technique was used the larger numbers resulted in a higher mortality as opposed to a lower mortality when immersion was practised. Again the small numbers gave the better fitting regression line.

A further investigation of the effect of the number of flies per container was attempted, using the standard containers normally loaded with 15 flies each. Tests were carried out at two concentrations of nicotine, 1.1 and 1.5% (used as nicotine sulphate by the spray technique) using 150 flies for each test, in lots of 5, 10, 15, and 30 flies per container. The larger containers used in the early tests when 150 flies were treated per container, were included with lots of 75 and 150 flies. The tests were replicated eight times (Table XII).

The steady rise in mortality as the numbers of test animals were increased would seem highly significant. Unfortunately any attempt to draw the probit lines for each test is bound to be disappointing as the slopes of such lines, determined from only two points are highly inaccurate. Thus the slopes determined from the above values vary widely. It would seem probable that determined from several points such lines might lie almost parallel. This, however, could not be tested as it was found impossible to carry out enough tests to determine five points or more on each of a number of curves

each day. It will be noted, however, that in Fig. 5B such parallelism is evident.

The rising mortality figures for any one treatment seem to bear an almost logarithmic relation to the increase in test animals per container. If this rise be graphed by plotting the numbers of flies on the abscissa against the mortalities on the ordinate the curve secured resembles the curves of absorption phenomena. Substituting the logarithms of the numbers of flies in the above graph does not give a completely straightened curve.

Effect of the Medium Used to Rear the Test Animal

In view of the fact that different experimenters are known to be using different media for rearing test animals and in view of the known influence of nutrition on susceptibility, a brief comparison was made of the susceptibility of *Drosophila* reared on banana, as used by the writer, and those reared on the potato–yeast medium suggested by Stultz (34). The tests were made with nicotine sulphate and the spray technique in the standard fashion. They were replicated five times. The results are shown in Table XIV.

The higher mortality in the check when the potato–yeast medium was used was due to a very high kill in the checks of two replicates. In still other tests replications were discarded because yeast, probably carried on the insect bodies, fermented the honey soaked plugs of vials in which flies were held previous to the mortality counts and resulting gases appeared to have anaesthetized the flies. Some difficulty was encountered in keeping available food plentiful in potato–yeast cultures and hence the flies of normal size. There are two factors involved, larval nutrition, which, if inadequate, results in undersized flies many of which may escape through the tulle covering containers, and adult nutrition, which we can only estimate from the mortalities, especially in the checks. Later it became necessary owing to a shortage in bananas, to use the potato–yeast medium to rear flies used in the tests recorded under in the following sections.

Variation Due to Different Experimenters

It is essential that a standardized toxicity test should give comparable results in the hands of different experimenters. To test this for the spray technique used, five workers were selected, the writer, a postgraduate student who had for some time previously assisted in this work, two fourth year students in entomology, and a girl studying the short course in household science and without previous laboratory training or experience. Nicotine sulphate was used in two concentrations (1.1% and 1.5% nicotine) and checks were tested with distilled water. Each experimenter tested each concentration on 10 containers of 15 flies each, each day. Eight replications were performed. The percentage mortalities obtained are recorded in Table XIII. The mortality data were transformed to the angular conversion values of Bliss's table and analysed by analysis of variance with the following results.

	Sum of squares	Degrees of freedom	Variance	F	F for 5% P	F for 1% P
Different workers	411.67	4	102.92	2.32	2.52	3.65
Different treatments	1100.68	1	1100.68	24.85	4.00	7.08
Different replications	1202.01	7	171.70	3.88	2.25	3.12
Interaction of workers and treatments	57.60	4	14.40			
Error	2790.89	63	44.29			
Total	5562.85	79				

It will be seen that the variance due to different workers, as shown in this test, might occur by chance in something more than 5% of such tests. There are probably some slight differences in mortalities as recorded by different workers but these are not as great as the differences due to different populations (replications) which are evidently significant. The interaction of workers and treatments is negligible. If the check cages, sprayed with water, are included as a treatment, to give another degree of freedom for treatments, and for the interaction, and 40 more for the total, the F value for treatment is greatly raised while that for workers becomes 2.31 and for replicates 3.66 indicating that the workers secured equally consistent results with treatments and check, while the check mortality varied less from population to population than did the mortalities with nicotine treatments. For this last reason it is deemed incorrect to include the water treatment in the analysis of variance as, compared to the nicotine treatments, it would not seem to be subject to equal chances of error.

Effect of Using Different-Sized Containers

The evidence secured by using different numbers of flies in a container, coupled with the two sizes of containers used when samples of 15 and 150 flies were compared, suggested a consideration of the possible effects of different-sized containers. To secure evidence on this point containers were prepared as in the earlier experiments but from glass tubing of five different inside diameters, viz., 9, 12, 14, 17, and 22 mm.

All containers were about 45 mm. long. Tests were then conducted using nicotine sulphate as a spray at two concentrations (1.1 and 1.5% nicotine) and a water check. Fifteen flies were used per container and all sizes were tested each day at each concentration. Eight replications were tested. The average percentage mortalities are given in Table XV. The following are the results of an analysis of variance performed on the angular mortality values.

	Sum of squares	Degrees of freedom	Variance	F	F for 5% P	F for 1% P
Different diameters	2841.79	4	710.45	16.35	2.52	3.65
Different replications	939.91	7	134.27	3.09	2.25	3.12
Different treatments	1003.02	1	1003.02	23.08	4.00	7.08
Interaction of diameters and treatments	320.32	4	80.08	1.84	2.52	3.65
Error	2737.73	63	43.46			
Total	7842.77	79				

It is evident that there is a significant difference in the observed mortalities obtained with different-sized containers. Inspection of the mortalities shows the greatest differences to occur between mortalities observed with the greatest and smallest diameters tested as compared with the three median sizes grouped together. The interaction of diameter and treatment is not significant but when we include the water treatment in our analysis this interaction reaches 2.99 (the F for 1% P for 60, and 8 degrees of freedom is 2.82). This is as we would expect since the check mortalities were unaffected by the container diameter. Again it seems that the water spray cannot be considered as a "treatment".

The causes of this variation resulting from container diameter remain to be investigated. The trend of the data suggests a parallelism between these and the results from lessening the number of flies in containers of constant size. Such an effect might be due to crowding (number of flies per unit of space), or to number of flies per unit of container surface area. Or it may be the effect of the diameter on the distribution and deposit of spray, as Potter (32) has shown such an effect when he varied the size of his spray tower.

This experiment is inadequate to finally determine the best container diameter to use. The best size, however, would seem to be that one by which day to day variation (due to population differences plus size and other factors) is least. An indication of the best of the sizes tested can thus be secured by treating each of the tests with each size as a separate experiment. Using the total mortalities for both concentrations for each day (expressed in angles) as normal variates, and determining the standard deviations for each, we arrive at the following:

Diameter of container, mm.	9	12	14	17	22
Standard deviation of results	1.97°	7.08°	3.83°	2.16°	2.21°

Within the limits of this experiment, which are recognized as very narrow, containers of a diameter of 9 mm. appear to give the most consistent results, while those of diameter 14 mm., used throughout all other tests, gave the second highest variation. It is proposed to further investigate this problem.

In general, it may be pointed out that all values obtained in toxicity tests to date, though functions of the type of insecticide and dosage, are not functions of these factors alone but are so definitely, also, functions of the test animal and technique used, that their value is purely relative and very limited. Thus a numerical *L.D.* 50 means nothing except in the light of the technique used. Only when one best technique, standardized in all its details, is used for all tests can results be compared accurately and even then the factors of different susceptibility of populations and of different test animals will remain.

Acknowledgments

As previously stated the data discussed in this paper have been accumulated since 1934 by a number of workers at Macdonald College, all under the direction of Dr. W. H. Brittain. Credit has been given for the various contributions in design of the apparatus, etc., made by Dr. J. McB. Cameron, Dr. M. L. Prebble, F. Cannon, and others, in the text and in the bibliography. The writer owes his gratitude to Dr. Brittain for continued assistance based on his invaluable experience; to Mr. Whitehead for the lettering on Illustration 13; and to Prof. Summerby, Macdonald College, and Dr. J. W. Hopkins, National Research Council, for help and suggestions with regard to the statistical analysis.

References

1. Bartlett, M. S. Suppl. J. Roy. Stat. Soc. 3 : 68-78. 1936.
2. Bartlett, M. S. Suppl. J. Roy. Stat. Soc. 3 : 185-194. 1936.
3. Bliss, C. I. Science (n.s.), 79 : 38-39, 409-410. 1934.
4. Bliss, C. I. Ann. Applied Biol. 22 : 134-167. 1935.
5. Bliss, C. I. Ann. Applied Biol. 22 : 307-333. 1935.
6. Bliss, C. I. Plant Protection Fasc. (Lenin Academy of Agricultural Science, Institute for Plant Protection) 12: 67-77. 1937.
7. Bliss, C. I. Ohio J. Sci. 38 : 9-12. 1938.
8. Bliss, C. I. Ann. Applied Biol. 26 : 585-615. 1939.
9. Cameron, J. McB. and Prebble, M. L. Project (unpublished). 1934.
10. Campbell, F. L. and Sullivan, W. N. Soap, 14 (6) : 119-125, 149. 1938.
11. Cannon, F. Unpublished M.Sc. Thesis. 1939.
12. Clark, A. and Leonard, W. H. J. Am. Soc. Agron. 31 : 55-66. 1939.
13. Cochran, W. G. Empire J. Exptl. Agr. 6 : 157-175. 1938.
14. Craufurd-Benson, H. J. Bull. Entomol. Research, 29 : 41-56. 1938.
15. Craufurd-Benson, H. J. Bull. Entomol. Research, 29 : 119-123. 1938.
16. Eagleson, C. Soap, 16 (7) : 96-99, 117. 1940.
17. Fisher, R. A. and Yates, F. Statistical tables for biological, agricultural and medical research. Oliver and Boyd, Ltd., Edinburgh and London. 1938.
18. Gaddum, J. H. Med. Research Council (Brit.), Special Rept. Series, No. 183. 1933.
19. Gilbert, H. A. and Marshall, J. Dept. Project (unpublished). 1932.
20. Hansberry, R. and Chiu, S. F. J. Econ. Entomol. 33 : 139-141. 1940.
21. Hemmingsen, A. M. Quart. J. Pharm. Pharmacol. 6 : 39-80, 187-218. 1933. (Cited in Bliss (4).)
22. Huber, L. L. and Sleesman, J. P. J. Econ. Entomol. 28 : 70-76. 1935.
23. Irwin, J. O. Suppl. J. Roy. Stat. Soc. 4 : 1-48; discussion, 49-60. 1937.
24. Maxwell, C. W. B. Unpublished M.Sc. Thesis, Macdonald College Library. 1939.
25. Maxwell, C. W. B. and Lord, F. T. Ann. Rept. Entomol. Soc. Ontario, 68 : 33-36. 1937.

26. MORLEY, P. M. Unpublished report, in Macdonald College Library. 1938.
27. O'KANE, W. C., GLOVER, L. C., and BLICKLE, R. L. New Hampshire Agr. Expt. Sta. Tech. Bull. 76. 1941.
28. O'KANE, W. C., WALKER, G. L., GUY, H. G., and SMITH, O. J. New Hampshire Agr. Expt. Sta. Tech. Bull. 54. 1933.
29. O'KANE, W. C., WESTGATE, W. A., and GLOVER, L. C. New Hampshire Agr. Expt. Sta. Tech. Bull. 58. 1934.
30. O'KANE, W. C., WESTGATE, W. A., GLOVER, L. C., and LOWRY, P. R. New Hampshire Agr. Expt. Sta. Tech. Bull 39. 1930.
31. PHILLIPS, A. M. and SWINGLE, M. C. J. Econ. Entomol. 33 : 172-176. 1940.
32. POTTER, C. Ann. Applied Biol. 28 : 142-169. 1941.
33. SHEPARD, H. H. and RICHARDSON, C. H. J. Econ. Entomol. 24 : 905-914. 1931.
34. STULTZ, H. T. Ann. Rept. Entomol. Soc. Ontario, 70 : 72-80. 1939.
35. TATTERSFIELD, F. Ann. Applied Biol. 21 : 691-703. 1934.
36. TATTERSFIELD, F. Ann. Applied Biol. 26 : 365-384. 1939.
37. TATTERSFIELD, F. and GIMINGHAM, C. T. Ann. Applied Biol. 14 : 217-239. 1927.
38. TATTERSFIELD, F. and MARTIN, J. T. Ann. Applied Biol. 25 : 411-429. 1938.
39. TATTERSFIELD, F. and MORRIS, H. M. Bull. Entomol. Research, 14 : 223-233. 1924.
40. TREVAN, J. W. Proc. Roy. Soc. (London) B, 101 : 483-514. 1927.
41. TUMA, V. Soap, 14 (6) : 109-111, 113, 115, 117, 151. 1938.

Note: Tables will be found on pages 61 to 75.

TABLE Ia

DAILY DATA SHEET. INSECTS (*Drosophila*) KILLED BY A SPRAY TREATMENT WITH NICOTINE SULPHATE IN A 1% SAPONIN SOLUTION. ONE REPLICATION. MAY 17, 1938

Container	Water		Saponin, 1%		Nicotine, % conc. 0.01		0.05		0.10		0.15		0.20		0.30		0.35		0.40		0.45		0.50		0.55		0.60		0.70		0.80		0.95		1.10		1.25		1.40		2.00	
	Number of animals alive (*A*) and dead (*D*)																																							
	A	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*	*A*	*D*
1	12	0	12	5	3	8	14	4	15	3	12	7	8	6	7	8	5	11	4	11	3	4	2	16	6	6	0	20	0	15	0	16	0	16	0	11	0	15	0	15	0	14
2	10	0	18	0	19	1	10	2	8	4	5	12	9	5	5	6	3	11	0	14	0	13	6	13	2	7	3	15	3	18	0	16	2	16	0	13	0	12	0	15	0	12
3	24	0	9	0	14	0	9	1	12	2	16	2	14	10	0	11	3	10	0	12	3	12	1	10	1	8	3	16	2	10	0	11	0	14	1	14	0	14	0	16	0	15
4	20	2	14	1	17	3	11	6	15	3	11	8	7	6	8	5	3	10	2	13	3	11	4	9	2	16	2	12	1	15	0	14	2	13	0	14	0	12	0	15	0	13
5	15	0	13	1	14	0	13	1	9	2	9	10	6	7	12	13	1	15	4	4	2	9	2	11	4	13	6	10	0	15	0	12	0	11	0	20	0	18	0	11	0	17
6	12	0	18	2	10	1	13	2	11	1	7	8	7	7	5	8	1	9	0	17	4	9	0	12	2	14	0	13	3	12	0	19	1	17	0	13	0	14	0	13	0	10
7	16	0	14	0	15	0	11	0	10	2	14	6	13	5	1	10	7	8	5	10	6	11	2	12	2	14	4	16	0	17	0	10	1	12	0	25	1	18	0	15	0	10
8	13	0	14	0	11	2	17	3	7	5	1	10	10	7	9	11	4	11	5	7	4	14	2	12	2	15	0	18	0	16	0	16	1	17	1	13	0	15	0	12	0	13
9	10	0	7	3	13	1	19	1	15	1	13	5	10	4	9	10	6	11	1	15	1	18	5	8	0	15	0	15	0	13	2	15	1	15	1	18	0	16	0	21	0	18
10	15	0	9	1	15	1	11	0	12	0	6	5	8	6	1	13	2	16	3	12	6	6	3	16	1	12	2	17	1	13	0	12	0	13	0	16	0	13	0	15	0	13
Totals	147	2	128	13	131	17	128	20	114	23	94	73	92	63	57	95	35	112	24	115	32	107	27	119	22	120	20	152	10	144	2	141	8	144	3	157	1	151	0	148	0	135
% Dead	1.34		9.22		11.49		13.51		16.79		43.71		39.87		62.51		76.26		82.73		76.98		81.51		84.51		88.37		91.56		98.60		94.74		98.13		99.34		100.00		100.00	

TABLE Ib

INSECTS (*Drosophila melanogaster*) KILLED BY APPLICATION OF A SPRAY TREATMENT (1% NICOTINE SULPHATE IN 1% SAPONIN). SUMMARIZED DATA FROM ONE EXPERIMENT SHOWING AVERAGE PERCENTAGE MORTALITIES FOR EACH OF 10 REPLICATES

Date	Water	Saponin, 1%	Nicotine, % conc.																	
			0.01	0.05	0.10	0.15	0.20	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.70	0.80	0.95	1.10	1.25	1.40	2.00
			Percentage dead																		
May 11	0.0	4.1	5.2	8.5	13.0	22.8	35.3	51.7	62.4	62.4	66.0	69.7	85.5	77.8	83.3	92.7	95.8	98.7	98.0	100.0	100.0
May 14	0.0	5.3	8.7	9.2	18.2	32.4	29.3	64.8	79.6	75.6	77.6	78.4	90.6	94.1	93.6	97.1	97.6	95.7	98.8	100.0	100.0
May 17	1.3	9.2	11.5	13.5	16.8	43.7	39.9	62.5	76.3	82.7	77.0	81.5	84.5	88.4	91.6	98.6	94.7	98.1	99.3	100.0	100.0
May 19	0.7	6.3	5.2	6.1	14.5	20.3	41.3	60.9	70.9	66.1	80.6	75.5	87.6	86.0	94.5	95.1	97.8	97.1	98.7	100.0	97.6
May 21	0.0	6.0	5.2	3.3	18.0	13.8	24.0	57.8	60.9	59.3	70.0	65.4	74.8	79.1	87.6	91.3	95.0	95.2	95.6	99.3	100.0
May 26	0.0	2.7	1.2	1.2	8.5	21.2	27.0	37.9	54.1	51.8	62.3	72.0	74.0	79.8	72.8	91.1	93.7	92.5	90.8	99.4	100.0
May 28	0.0	2.7	2.5	6.6	13.0	17.4	22.2	41.3	45.4	49.0	47.0	53.6	61.2	71.1	74.5	86.2	95.4	97.4	95.3	99.5	100.0
May 31	1.2	8.1	10.2	13.0	19.1	24.0	32.7	59.3	65.9	69.9	70.0	70.2	87.3	89.0	83.3	89.1	93.4	99.5	99.5	98.4	100.0
June 2	1.0	4.5	2.1	4.9	17.3	11.1	25.0	34.9	44.2	63.1	64.1	66.3	79.1	71.4	70.0	73.7	89.8	95.1	98.7	97.1	98.8
June 4	0.0	4.7	5.5	11.2	11.9	20.0	40.0	39.0	58.5	47.0	62.2	61.5	75.0	71.0	79.4	87.9	89.6	88.9	96.8	95.4	96.2

TABLE Ic

AVERAGE LOG OF DOSAGE ($\bar{x}$), AVERAGE PROBIT ($\bar{y}$), SLOPE OF THE REGRESSION LINE (b), AND CHI² FOR EACH OF 10 REPLICATIONS USING NICOTINE SULPHATE IN A 1% SAPONIN SOLUTION AS SPRAY ON *Drosophila melanogaster*

Replicate	$\bar{x}$	$\bar{y}$	b	χ^2
1	1.62	5.47	3.019	26
2	1.58	5.68	2.955	89
3	1.59	5.73	2.835	31
4	1.60	5.64	3.265	23
5	1.64	5.44	3.213	21
6	1.68	5.44	3.138	49
7	1.69	5.32	3.341	58
8	1.64	5.60	3.057	49
9	1.69	5.40	3.066	50
10	1.69	5.44	2.550	36

TABLE Id

AVERAGE MORTALITY OF *Drosophila melanogaster*, OBTAINED FROM 4, 6, 8, AND 10 REPLICATES USING NICOTINE SULPHATE IN A 1% SAPONIN SOLUTION. ERRORS OF THESE EXPERIMENTS ARE SHOWN AS SECURED BY ANALYSIS OF VARIANCE AND ARE EXPRESSED IN ANGLES OF EQUAL INFORMATION

	Nicotine, % conc.																		
	0.01	0.05	0.10	0.15	0.20	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.70	0.80	0.95	1.1	1.25	1.4	2.0
Four replicates																			
No. killed, %	7.37	9.34	15.56	29.80	36.44	62.45	72.25	71.70	82.79	76.05	87.03	86.52	90.75	98.37	96.25	97.39	98.70	100.00	99.39
Angles (average)	15.9	17.8	23.4	32.8	37.0	50.9	58.4	58.1	60.4	61.1	69.4	68.9	73.0	79.0	79.9	81.2	83.7	90.0	88.0
Standard error of a mean, 1.64°.																			
Variance:																			
Between replicates, 104.53.																			
Between treatments, 2267.09.																			
Error, 10.82.																			
Six replicates																			
No. killed, %	6.15	6.98	14.95	25.87	32.97	57.26	67.18	66.33	77.20	73.61	84.24	82.49	87.25	94.31	95.61	96.20	96.87	99.61	99.39
Angles (average)	13.7	14.5	22.7	30.1	34.8	48.6	55.4	54.8	58.4	59.4	66.1	66.9	70.1	76.8	78.7	79.4	81.0	88.1	89.0
Standard error of a mean, 1.02°.																			
Variance:																			
Between replicates, 277.05.																			
Between treatments, 3494.24.																			
Error, 6.29.																			
Eight replicates																			
No. killed, %	6.15	7.69	15.35	24.58	31.66	55.90	64.29	64.61	72.55	70.68	80.67	81.88	85.16	92.64	95.31	96.76	97.01	99.51	99.51
Angles (average)	13.8	15.4	22.9	29.3	34.0	47.7	53.6	53.8	56.3	57.5	64.6	66.2	68.3	74.0	78.0	80.6	81.6	87.6	89.0
Standard error of a mean, 1.21°.																			
Variance:																			
Between replicates, 274.57.																			
Between treatments, 4602.17.																			
Error, 11.73.																			
Ten replicates																			
No. killed, %	6.72	7.76	15.00	22.67	31.67	51.00	61.82	62.69	67.67	68.43	79.96	80.76	84.17	89.27	94.29	95.81	97.12	98.90	99.26
Angles (average)	13.29	15.56	22.76	28.03	34.08	45.63	52.03	52.58	55.56	56.65	63.97	64.44	66.56	72.87	76.73	79.42	81.72	85.76	87.47
Standard error of a mean, 1.45°.																			
Variance:																			
Between replicates, 335.48.																			
Between treatments, 5502.96.																			
Error, 21.09.																			

TABLE Ie

AVERAGE MORTALITIES (EXPRESSED AS PERCENTAGES) OBSERVED BY CAMERON AND PREBBLE (9) WHEN THEY SPRAYED ADULT *Drosophila melanogaster* WITH DIFFERENT CONCENTRATIONS OF NICOTINE SULPHATE IN 1% SAPONIN SOLUTION

	Nicotine, % conc.																	
	0.025	0.05	0.075	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.60	0.70	0.80	0.90	1.10	1.20	1.40
No. killed, %	4.85	8.61	16.3	17.1	23.3	38.1	34.3	40.5	51.7	62.0	63.8	72.3	78.0	79.9	90.2	92.8	94.9	96.1	96.6
Standard error	1.11	2.45	2.49	2.66	2.75	4.34	2.33	2.37	2.54	3.82	3.36	3.23	3.43	3.51	3.47	3.17	3.41	2.38	2.87
No. of containers tested	87	69	81	65	50	49	81	112	83	69	62	72	65	55	56	39	31	29	35

TABLE II

MORTALITY OF *Drosophila melanogaster*, OBTAINED WITH "NICOFUME" USED AS A SPRAY. (MORTALITY IS EXPRESSED IN PERCENTAGES AND ANGLES OF EQUAL INFORMATION. THE STANDARD ERROR, SECURED BY ANALYSIS OF VARIANCE, IS IN ANGLES OF EQUAL INFORMATION.) (10 REPLICATES)

	Nicotine, % conc.																		
	0.00	0.05	0.075	0.10	0.125	0.150	0.175	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.70	0.80	1.00
No. killed, %	0.45	2.24	4.85	12.68	18.73	25.29	34.61	34.70	50.94	57.78	68.32	79.87	87.03	89.96	94.11	96.40	98.30	99.99	99.94
Angle	—	8.1	11.86	20.13	25.92	30.55	36.54	36.13	45.32	49.47	55.98	63.47	69.64	72.47	76.68	79.64	83.52	87.29	89.43

Standard error of a mean, 1.25°.
Variance:
Between replicates, 137.15.
Between treatments, 7126.83.
Error, 15.72.

TABLE III*a*

MORTALITY OF *Drosophila melanogaster* OBTAINED BY THE SPRAY TECHNIQUE USING "NICOFUME" IN A 1% SAPONIN SOLUTION (10 REPLICATES)

	Nicotine, % conc.																			
	0.00	0.01	0.025	0.050	0.075	0.10	0.125	0.150	0.175	0.20	0.225	0.250	0.30	0.35	0.40	0.45	0.50	0.60	0.75	0.90
No. killed, %	0.61	3.67	4.75	4.32	7.74	9.73	10.58	19.45	23.59	28.20	33.90	43.75	48.89	52.25	64.02	60.09	77.50	87.14	92.76	98.06
Angle	—	10.03	11.67	11.43	14.55	17.48	18.52	26.03	28.90	31.65	33.66	40.15	43.87	47.91	53.38	—	62.81	69.83	75.43	82.90

Standard error of a mean, 1.33°.
Variance:
Between replicates, 489.16.
Between treatments, 6944.93.
Error, 17.70.

TABLE III*b*

AVERAGE MORTALITIES (EXPRESSED AS PERCENTAGES) OBSERVED BY CAMERON AND PREBBLE (9) WHEN THEY SPRAYED *Drosophila melanogaster* WITH DIFFERENT CONCENTRATIONS OF "NICOFUME" IN A 1% SAPONIN SOLUTION

	Nicotine, % conc.										
	0.01	0.025	0.05	0.075	0.10	0.125	0.150	0.20	0.25	0.30	0.40
No. killed, %	4.21	3.74	4.75	12.61	25.21	45.90	63.26	66.05	80.90	85.64	95.04
Standard error	± 3.16	1.00	3.60	1.40	4.06	0.53	2.99	3.97	3.97	3.18	3.59
No. of containers tested	82	92	175	176	177	210	251	90	85	85	92

TABLE IV*a*

MORTALITY OF *Drosophila melanogaster* OBTAINED BY THE IMMERSION TECHNIQUE USING NICOTINE SULPHATE (10 REPLICATES). (STANDARD CONTAINERS LOADED WITH 15 FLIES EACH WERE USED)

	Nicotine, % conc.																				
	0.00	0.20	0.25	0.30	0.40	0.50	0.60	0.70	0.80	0.90	1.0	1.1	1.2	1.3	1.4	1.6	1.8	2.0	2.5	3.0	4.0
No. killed, %	3.84	9.2	13.93	24.76	37.10	44.63	48.27	59.60	67.01	70.45	75.10	73.91	83.08	80.89	88.59	85.41	94.40	90.00	95.17	96.38	99.68
Angle	—	19.68	23.50	32.13	38.62	43.63	45.40	51.09	56.63	57.96	61.72	60.70	67.95	65.54	71.87	69.27	77.60	74.05	78.67	80.26	88.29

Standard error of a mean, 2.17°.
Variance:
Between replicates, 389.23.
Between treatments, 3734.60.
Error, 47.47.

TABLE IV*b*

MORTALITY OF *Drosophila melanogaster* OBTAINED USING NICOTINE SULPHATE BY THE IMMERSION TECHNIQUE AND TREATING CONTAINERS OF 150 FLIES EACH. (PERCENTAGE VALUES ARE GIVEN FOR EACH REPLICATE)

	Nicotine, % conc.																				
	0.0	0.20	0.25	0.30	0.40	0.50	0.60	0.70	0.80	0.90	1.0	1.1	1.2	1.3	1.4	1.6	1.8	2.0	2.5	3.0	4.0
No. killed, %																					
Repl. 1	1	6	7	9	7	15	18	30	52	50	62	66	80	71	75	91	75	62	96	98	100
Repl. 2	1	1	2	2	5	17	18	3	9	17	21	15	31	25	17	25	66	48	50	78	99
Repl. 3	1	6	3	1	4	10	10	10	11	17	16	19	13	19	33	64	70	62	74	66	92
Repl. 4	0	0	3	1	4	3	7	8	12	12	17	26	39	31	30	57	53	72	43	87	95
Repl. 5	0	0	1	1	7	12	5	10	15	5	16	26	20	21	32	37	66	25	58	84	98
% of total	0.6	3.35	3.65	2.65	5.18	11.77	11.48	11.20	23.28	17.39	25.18	30.20	33.24	31.52	39.57	53.54	58.76	58.00	61.76	84.48	97.17
Angle	—	6.82	8.10	8.54	13.30	18.76	19.36	19.28	25.22	25.40	30.16	32.86	36.70	34.72	37.42	48.42	54.42	47.16	54.68	66.70	81.18

Standard error of a mean, 3.13°.

TABLE V

MORTALITY OF *Drosophila melanogaster* OBTAINED BY THE IMMERSION TECHNIQUE USING NICOTINE SULPHATE IN 1% SAPONIN SOLUTION (10 REPLICATES)

	Nicotine, % conc.																
	0.00	0.10	0.20	0.25	0.30	0.35	0.40	0.50	0.60	0.70	0.80	0.95	1.25	1.50	2.00	3.00	4.00
No. killed, %	1.02	1.02	2.41	5.70	5.33	10.32	12.60	20.26	27.06	39.32	41.61	48.75	63.41	68.96	75.21	89.07	90.75
Angle	—	4.91	8.97	13.16	13.33	18.66	20.13	26.42	30.99	38.96	40.26	43.82	53.30	56.39	60.81	69.55	73.04

Standard error of a mean, 1.39°.
Variance:
Between replicates, 267.63.
Between treatments, 4869.31.
Error, 19·08.

TABLE VI*a*

MORTALITY OF *Drosophila melanogaster* OBTAINED BY THE IMMERSION TECHNIQUE USING "NICOFUME" (10 REPLICATES)

	Nicotine, % conc.																
	0.0	0.05	0.075	0.10	0.125	0.15	0.175	0.20	0.225	0.25	0.30	0.35	0.40	0.45	0.50	0.60	0.90
No. killed, %	3.17	3.79	9.12	12.09	17.14	22.43	35.83	34.62	45.13	48.34	71.49	60.98	83.03	87.70	88.33	96.61	99.56
Angle	—	15.03	19.77	22.44	25.85	29.57	35.24	37.52	42.84	45.26	58.46	52.96	67.05	70.65	70.90	80.55	87.15

Standard error of a mean, 1.86°.
Variance:
Between replicates, 224.25.
Between treatments, 5269.60.
Error, 35.20.

TABLE VI*b*

MORTALITY OF *Drosophila melanogaster* OBTAINED BY MORLEY (26) USING THE IMMERSION TECHNIQUE AND "NICOFUME"

	Nicotine, % conc.									
	0.0	0.2	0.3	0.4	0.5	0.6	0.7	0.8	1.0	1.5
No. killed, %	0.5	4.2	9.7	21.9	32.9	50.8	65.1	74.9	86.3	98.2
Standard error	—	0.03	0.11	0.45	0.70	1.34	1.62	2.2	1.4	0.85
Number of containers tested	70	60	70	70	70	50	50	40	50	30

TABLE VII*a*

MORTALITY OF *Drosophila melanogaster* OBTAINED BY THE IMMERSION TECHNIQUE USING "NICOFUME" IN A 1% SAPONIN SOLUTION (10 REPLICATES)

	Nicotine, % conc.															
	0.00	0.05	0.075	0.10	0.125	0.150	0.175	0.20	0.225	0.250	0.30	0.35	0.40	0.50	0.60	0.90
No. killed, %	3.67	1.49	4.26	9.26	17.86	26.29	37.16	46.49	48.12	53.84	66.96	76.96	83.76	90.87	98.32	100.00
Angle	—	12.91	15.78	20.54	26.98	32.79	39.16	44.16	44.91	48.52	56.46	61.69	67.91	73.89	84.04	90.00

Standard error of a mean, 1.60°.
Variance:
Between replicates, 90.97.
Between treatments, 5848.53.
Error, 24.78.

TABLE VII*b*

MORTALITY OF *Drosophila melanogaster* OBTAINED BY CAMERON AND PREBBLE (9) USING THE IMMERSION TECHNIQUE AND "NICOFUME" IN A 1% SAPONIN SOLUTION

	Nicotine, % conc.										
	0.01	0.025	0.05	0.075	0.10	0.125	0.15	0.20	0.25	0.30	0.40
No. killed, %	10.56	6.01	8.52	17.44	32.02	41.92	53.41	47.27	65.66	79.15	96.41
Standard error	6.33	0.98	8.04	2.50	3.18	2.39	3.18	2.48	2.33	2.72	0.90
Number of containers tested	91	92	32	32	32	92	92	91	92	89	88

TABLE VIII*a*

MORTALITY OBTAINED USING THE SPRAY TECHNIQUE AND NICOTINE SULPHATE IN A 1% SAPONIN SOLUTION ON FLIES (*Drosophila melanogaster*) OF DIFFERENT AGES (10 REPLICATES)

	Age of flies														
	1 day			2 days			3 days			4 days			5 days		
	Nicotine, % conc.														
	0.20	0.30	0.50	0.20	0.30	0.50	0.20	0.30	0.50	0.20	0.30	0.50	0.20	0.30	0.50
No. killed, %	3.92	10.08	19.07	3.56	6.27	16.54	3.04	3.85	14.66	2.72	3.81	12.15	4.04	6.03	18.22
Kill due to water, %	0.15			0.17			0.00			0.17			0.00		

TABLE VIII*b*

MORTALITY OBTAINED USING THE SPRAY TECHNIQUE AND NICOTINE SULPHATE ON FLIES (*Drosophila melanogaster*) OF DIFFERENT AGES (10 REPLICATES)

	Age of flies			
	1 day	2 days	3 days	4 days
	Nicotine, % conc.			
	1.1	1.1	1.1	1.1
No. killed, %	51.3	41.6	45.7	36.4

TABLE VIII*c*

MORTALITY OBTAINED BY CAMERON AND PREBBLE (9) USING NICOTINE SULPHATE AND 1% SAPONIN AS A SPRAY ON *Drosophila melanogaster* OF DIFFERENT AGES*

	Age of flies									
	Not over 3 days					Not over 4 days				
	Nicotine, % conc.									
	0.15	0.20	0.40	0 50	0.60	0.15	0.20	0.40	0.50	0.60
No. killed, %	7.7	20.5	51.3	64.7	68.2	23.3	38.1	62.0	72.3	78.0
Standard error	2.63	2.66	3.57	3.25	3.95	2.75	4.34	3.82	3.23	3.43
No. of containers treated	44	41	54	48	25	50	49	69	72	65

* *The percentage mortalities have been corrected by Abbott's formula, the corrections being based on the mortality obtained using 1% saponin alone.*

TABLE IX

MORTALITY OF *Drosophila melanogaster* OBTAINED BY THE SPRAY TECHNIQUE USING NICOTINE SULPHATE IN A 1% SAPONIN SOLUTION SPRAYED AT DIFFERENT RATES (EIGHT REPLICATES)

	Rate of spraying															
	1 cc. per 10 sec.								1 cc. per 25 sec.							
	Nicotine, % conc.															
	0.0	0.1	0.2	0.3	0.5	0.7	0.9	1.2	0.0	0.1	0.2	0.3	0.5	0.7	0.9	1.2
No. killed, %	1.62	6.96	16.8	21.1	42.4	58.6	68.2	76.1	1.47	6.26	14.5	22.7	43.4	51.0	61.1	74.5
Angle	—	14.3	22.7	27.3	39.4	48.4	54.2	60.4	—	13.5	21.2	27.6	41.1	45.2	49.9	59.9
	Standard error of a mean, 1.84°. Variance: Between replicates, 267.35. Between treatments, 2377.35. Error, 27.18.								Standard error of a mean, 1.61°. Variance: Between replicates, 222.26. Between treatments, 2224.98. Error, 20.72.							

TABLE X

MORTALITY OF *Drosophila melanogaster* OBTAINED BY THE SPRAY TECHNIQUE USING NICOTINE SULPHATE (10 REPLICATES)

	No. flies per container															
	15								150							
	Nicotine, % conc.															
	0.0	0.2	0.6	0.8	1.1	1.4	1.8	2.6	0.0	0.2	0.6	0.8	1.1	1.4	1.8	2.6
No. killed, %	0.85	9.44	31.40	39.70	56.60	63.80	77.70	88.80	1.81	28.15	64.8	79.8	88.6	91.6	96.8	98.8
Angle	—	16.28	35.05	40.70	48.88	54.74	62.51	72.32	—	30.47	52.58	63.17	70.09	73.76	80.04	84.95
	Standard error of a mean, 1.62°. Variance: Between replicates, 144.81. Between treatments, 3451.67. Error, 26.22.								Standard error of a mean, 2.24°. Variance: Between replicates, 141.78. Between treatments, 3461.29. Error, 49.59.							

TABLE XI

PERCENTAGE MORTALITY OF *Drosophila melanogaster* OBSERVED IN A COMPARISON OF NICOTINE SULPHATE WITH AND WITHOUT DIFFERENT CONCENTRATIONS OF SAPONIN, USING THE SPRAY TECHNIQUE (EIGHT REPLICATES)

	Nicotine, %								
	0			1.1			1.5		
	Saponin, %								
	0	1	3	0	1	3	0	1	3
Mortality, %	0.93	5.37	7.88	38.81	70.95	76.66	55.06	80.33	82.50
Angle	4.32	13.05	15.95	38.31	57.56	60.96	47.96	64.06	65.97

Variance:
Between replicates, 38.62.
For interaction saponin × nicotine, 80.50.
Error, 25.24.

TABLE XII

THE EFFECT OF THE NUMBER OF FLIES (*Drosophila melanogaster*) TESTED IN A CONTAINER ON THE PERCENTAGE MORTALITY OBSERVED, WHEN A NICOTINE SULPHATE SPRAY IS USED (EIGHT REPLICATES)

No. flies per container	Nicotine, % conc.		
	0	1.1	1.5
	Mortality, expressed as % dead		
5	0.76	55.5	70.4
10	1.21	68.4	78.6
15	0.36	73.5	79.3
30	0.97	80.2	90.6
75	2.05	83.3	87.4
150	3.22	82.7	93.5

TABLE XIII

PERCENTAGE MORTALITY OBTAINED BY DIFFERENT EXPERIMENTERS TESTING NICOTINE SULPHATE SPRAY ON ADULT *Drosophila* (FIVE REPLICATES)

	Experimenter				
	A	*B*	*C*	*D*	*E*
Mortality, % With water	0.4	0.6	0.1	0.4	0.2
With 1.1% nicotine	42.9	47.9	38.4	52.8	43.1
With 1.5% nicotine	56.1	57.2	56.2	66.0	52.9

TABLE XIV

PERCENTAGE MORTALITY OBTAINED TESTING FLIES (*Drosophila melanogaster*) REARED ON BANANAS AND THOSE REARED ON POTATO–YEAST MEDIUM WITH NICOTINE SULPHATE SPRAY (EIGHT REPLICATES)

	Treatment, nicotine conc. %		
	0	1.1	1.5
	Mortality, %		
Flies reared on banana	0.52	35.48	60.46
Flies reared on potato–yeast	3.59	46.74	61.24

TABLE XV

PERCENTAGE MORTALITY OBTAINED WITH NICOTINE SULPHATE SPRAY TESTED ON EQUAL NUMBERS OF FLIES (*Drosophila melanogaster*) IN DIFFERENT-SIZED CONTAINERS (EIGHT REPLICATES)

	Container diameter, mm.				
	9	12	14	17	22
Mortality, %					
With water	1.1	1.0	0.7	1.3	0.2
With 1.1% nicotine	64.5	55.2	51.4	51.2	28.1
With 1.5% nicotine	69.7	62.2	64.3	69.3	47.0

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. APRIL, 1943 NUMBER 4

THE CARDIAC OUTPUT OF FOUR FRESHWATER FISH[1]

BY J. S. HART[2]

Abstract

The stroke output of the heart was determined by measurement of the amount of blood in the ventricle at systole and diastole in four species of fish, the bowfin, *Amia calva* L., the common sucker, *Catostomus commersonii* (Lacépède), the carp, *Cyprinus carpio* L., and the catfish, *Ameiurus nebulosus* (Le Sueur).

The stroke output varies with the size of the individual. Smaller individuals of a species, although possessing a smaller absolute output, have a larger output in relation to their size than do larger individuals.

Over the size range at which they can be compared (300 to 600 gm.) the four species differ in their stroke output. The catfish has the highest output and the sucker the lowest. The bowfin and the carp are intermediate in this respect. At 500 gm., the outputs of the catfish, bowfin, carp, and sucker per stroke are respectively 0.26 gm., 0.22 gm., 0.18 gm., and 0.11 gm.

The results of the present investigation were correlated with those of Black (Biol. Bull. 79 : 215-229. 1940); the output of the heart was found to correlate inversely with the effect of carbon dioxide on the blood of the same species, and directly with the affinity of the blood for oxygen. Differences in the circulation may compensate for differences in oxygen transport imposed by the varying effects of carbon dioxide on the blood.

Introduction

Previous work on oxygen transport in fish has been concerned largely with the physico-chemical properties of the blood. The extensive variations in these properties have indicated a great range in the possibilities for oxygen transport in fish. Often specific dissimilarities in properties of the blood have been related to differences in the external environment in which the fish live.

The properties of the blood of particular interest in this connection are the affinity for oxygen and the effect of carbon dioxide on the oxygen affinity. Krogh and Leitch (4) first noted that these properties varied greatly from species to species, a fact later demonstrated by Root (6), Willmer (7), and Black (1). In the four species studied here, the range of possibilities for oxygen transport by virtue of the carbon dioxide effect alone was pointed out by Black (1). He showed that the differences were such that if all other respiratory characteristics remained the same, the carbon dioxide sensitivity of the common sucker blood might enable it to transport about 15 times as

[1] *Manuscript received September 22, 1942.*

Contribution from the Ontario Fisheries Research Laboratory, Department of Zoology, University of Toronto, Toronto, Ont.

[2] *Graduate Student, Department of Zoology, University of Toronto.*

much oxygen as the blood of the catfish and four times as much as the carp for equal volumes of blood circulated. Such differences in the blood should confer on each species a distinction in the transport of oxygen according to its carbon dioxide sensitivity, and it was thought that these differences might be reflected in differences in ability of the fish to utilize oxygen. However, measure of the maximum rates of oxygen transport (2) showed that the yellow perch, which was sensitive to carbon dioxide, consumed oxygen at a lower rate than the catfish, which had blood tolerant to carbon dioxide. Hence it seemed evident that factors other than the properties of the blood were involved in transport of oxygen.

An investigation of the cardiac output and beat rate of the catfish, carp, bowfin, and common sucker was carried out in order to determine the oxygen transport capacity from the mechanical standpoint. Since, as shown by Black (1), the chemical aspects of the bloods of these four species were such as to confer large advantages to certain of them in the transport of oxygen, the same species were studied here to determine whether or not certain mechanical aspects of the circulation were more advantageous to some species than to others.

Method

The fish used were bought from live-fish dealers in Toronto during the months from December, 1939, to April, 1940. The catfish, bowfin, and carp were caught largely in Lake Erie, whereas most of the suckers were obtained from Lake Simcoe. Before being used, the fish were kept in tanks for various periods of time, from a few hours to a week, at approximately 10° C. The experiments were carried out on 48 catfish, 43 bowfins, 40 carp, and 37 suckers.

In preparation for an experiment, the fish was stunned by blows on the head and the beating heart was exposed by cutting through the ventral body wall and pericardium. This and subsequent operations were carried out at room temperature. Ligatures were then placed loosely around the bulbus arteriosus and atrioventricular passage flush with the ventricle. These were tightened to isolate the quantity of blood contained in the ventricle from the rest of the circulatory system. After ligation, the ventricle was removed and weighed with the contained blood. The ligatures and adhering portions of the atrium and bulbus were carefully trimmed off and their weight subtracted from the first weight. The ventricle was then opened, washed thoroughly, hardened in 10% formalin for several hours, squeezed dry, and weighed again. This procedure was adopted since it was found possible to remove from 30 to 60% more fluid from the spongy inner surface of the ventricle when so treated than could be squeezed from the fresh material without damage to it. It was found that wet ventricles weighed fresh and also after being hardened in formalin showed no significant difference in weight, so that hardening caused no change in weight of the muscle itself.

Four different methods were used for ligating the ventricle during diastole. The first consisted in tightening the two ligatures simultaneously in the

interval between the contraction of the atrium and the ventricle. In many fish a modification of the above technique was used; in this the atrial ligature was tightened following the next succeeding contraction. A third variation was sometimes used for suckers; in these the heart-beat rate was often high and it was necessary to insert a cannula into the bulbus to relieve the pressure before the ligatures were tied; this will be referred to later (page 80). Finally, the hearts were sometimes poisoned with potassium nitrate to cause them to stop beating in diastole (5). Except in the bowfin, all these methods of preparation gave similar results. The quantities of blood in the ventricles were greater when the heart of the bowfin was poisoned with potassium than when it was not; hence these values have not been considered in the average although they are plotted on the graph (Fig. 1).

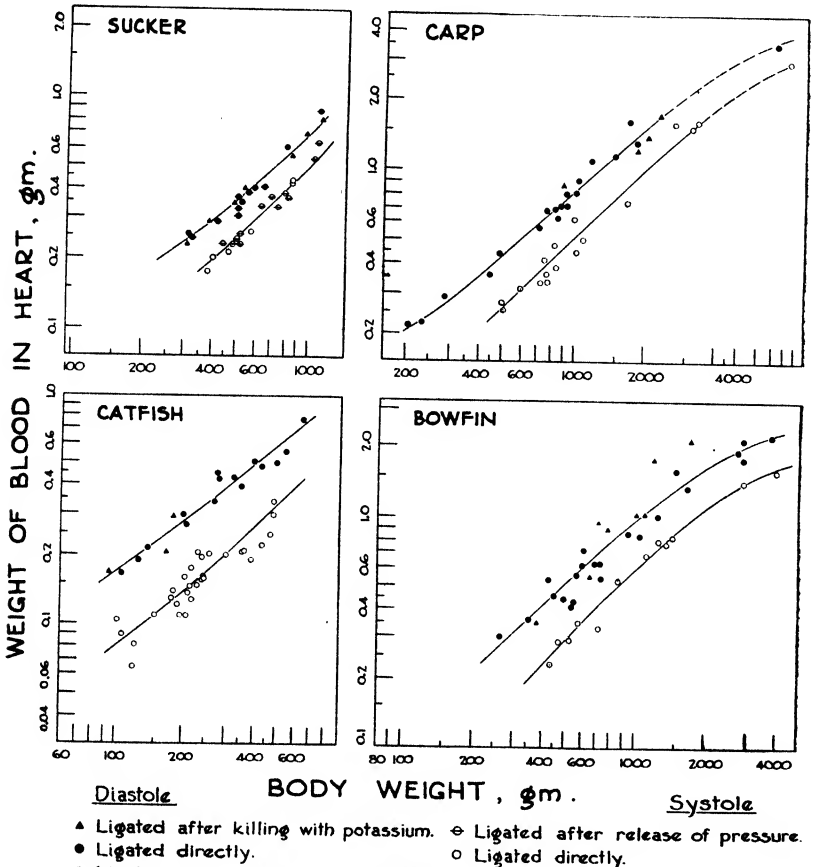


FIG. 1. *Curves showing the weight of the blood in the ventricle in diastole and systole of the bowfin, sucker, carp, and catfish as a function of body weight, double logarithmic grid.*

In measuring the blood content during systole, the ligatures were for the most part tied simultaneously, immediately following ventricular contraction. Occasionally the atrial ligature was tied one, two, or three beats before the bulbus ligature. In suckers with high beat rate the ventricles were ligated after release of pressure by the insertion of a cannula (see below). All these methods gave similar results.

There is a difference in the accessibility of the hearts of the four species. The hearts of the bowfin and the sucker are readily exposed, whereas the hearts of the catfish and carp are exposed with difficulty because of the presence of the bones of the pectoral girdle. In the latter two species more shock resulted than in the former two. The shock of exposing the heart was reflected in some fish by an acceleration in beat rate, in others by a retardation. In extreme cases, the exposure resulted in the cessation of the atrial beat for short periods; this was especially marked in the catfish.

The dynamics of the heart-beat are affected by various factors. It is well known that variations occur in the stroke output of the hearts of higher vertebrates with changes in beat rate. Such changes in rate commonly occurred in the fish hearts when they were being exposed, and when the ligatures were being placed in position for tying. In the sucker, the effect of stunning and of struggling on the part of the fish usually caused a marked acceleration of the beat rate from an average of 20 up to 35 or 40 beats per minute. In such fish, the bulbus and ventricle often became greatly distended with blood, and it was found that when ligatures were tied directly there was often more blood in the ventricle at systole than there was normally at diastole (i.e. when the rate was low). However, when a cannula was inserted into the bulbus to allow the blood excess to escape and relieve the pressure before the ligatures were tied, the results were similar to those obtained by the other methods. The rate of the heart-beat of a representative sample of the fish under the conditions of the study was determined and is recorded in Table I.

Results

In Fig. 1, the weight of the blood contained in the ventricle at diastole and at systole is plotted against body weight on a double logarithmic grid. Each point represents one fish. The difference between corresponding points on the diastolic and systolic curves for one species represents the weight of blood passed per beat, the stroke weight or *stroke output* under the experimental conditions. These differences are plotted against the body weight in Fig. 2. It may be seen that within each species, the stroke output increases as the body weight increases. The size range differed in the specimens used but the four species may be compared over the range from 300 to 600 gm. At 500 gm., the catfish has the largest cardiac output per beat, i.e., 0.26 gm. of blood (Table I); at this weight the bowfin has an output of 0.22 gm., the carp 0.18 gm., and the sucker 0.11 gm. Although the stroke output increases as the body weight increases, the output per unit weight decreases as the body weight increases (Fig. 3).

TABLE I

HEART-BEAT RATE, STROKE OUTPUT, MEAN MINUTE OUTPUT, AND VENTRICLE WEIGHT IN THE CATFISH, CARP, BOWFIN, AND SUCKER, AND COMPARISON WITH PRESSURES OF OXYGEN REQUIRED FOR HALF SATURATION AND WITH THE CARBON DIOXIDE EFFECT UPON BLOOD

	Catfish	Carp	Bowfin	Sucker
CO_2 effect, rise* in pO_2, in mm. Hg, due to 1 mm. CO_2	0.25	1.0	1.4	4.0
pO_2, in mm. Hg, at 50% HbO_2* with 0-2 mm. pCO_2	1.4	5.0	4.0	12.0
Stroke output, gm., at 500 gm. body wt.	0.26	0.18	0.22	0.11
Heart rate**, beats per minute	21.6 ± 4.3 (17 fish)	21.1 ± 3.1 (10 fish)	13.9 ± 2.5 (20 fish)	20.4 ± 1.3 (8 fish)
Mean minute output, gm., under experimental conditions	5.50	3.80	3.06	2.24
Ventricle weight, gm., at 500 gm. body weight	0.185	0.170	0.180	0.175

* *From Black (1).*

** *In the bowfin, catfish, and the carp the values for beat rate are recorded from fish selected at random. The beat rate for suckers is the mean after excluding the fish in which the bulbus and atrium were distended with blood.*

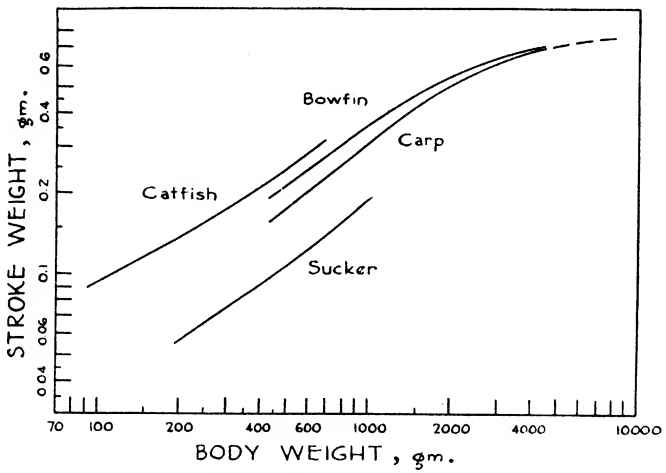


FIG. 2. *The relation between stroke output and body weight of the bowfin, sucker, carp, and catfish, double logarithmic grid. The stroke output was obtained by subtracting corresponding points on the systolic from the diastolic curves for each species in Fig. 1.*

The mean beat rate measured after exposure of the heart was found not to vary greatly from species to species (Table I). The values of the minute output shown in this table, obtained by multiplying the beat rate by the corresponding cardiac output per beat, are greatest for the catfish and are followed in turn by those of the carp, the bowfin, and the common sucker.

The cardiac output does not appear to be correlated with the mass of the ventricular muscle. At 500 gm. body weight there is little difference in the

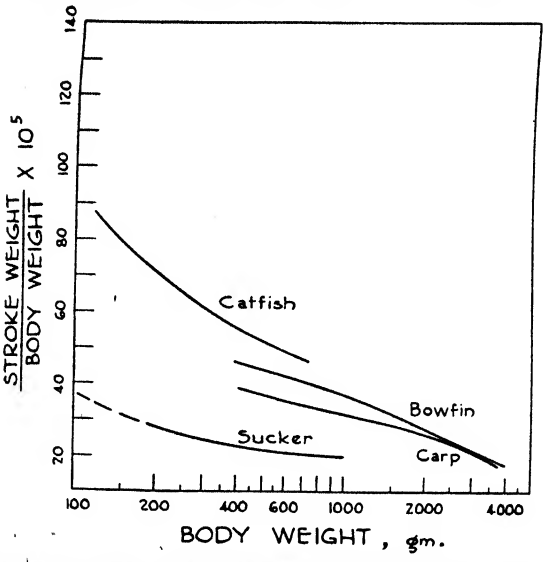


FIG. 3. *The relation between stroke output per unit weight and body weight of the bowfin, sucker, carp, and catfish, semilogarithmic grid.*

weight of the ventricle in each species although there are large differences in output (Table I). On the other hand, the differences in output appear to be related to the magnitude of the expansion and contraction of the ventricle through the cardiac cycle. Transverse sections indicate that during diastole the ventricle of the catfish has a larger cavity than that of the sucker (Figs. 4 and 5).

Discussion

Although the determinations were made under abnormal conditions on fish out of water, it is believed that the results obtained give a good approximation of the magnitude of the cardiac output in the four species. The hearts were usually beating regularly and circulating blood, and the regularity of the relation of blood weight to body weight at systole and diastole suggests the significance of the measurements as an indication of what may happen under normal conditions. The determination of the output under natural conditions was not the purpose of this investigation, but rather the determination of the specific differences as they existed under the experimental conditions.

The method described above has been applied to similar species in other localities and very similar results have been obtained. For example, both the catfish examined at Toronto and catfish investigated at Welaka, Florida (3), had a mean stroke output of 0.27 gm. of blood at 500 gm. body weight. Suckers taken from the Delaware River at Philadelphia in December, 1940, had a stroke output of 0.13 gm. of blood at this weight, the suckers examined at Toronto had an output of 0.11 gm.

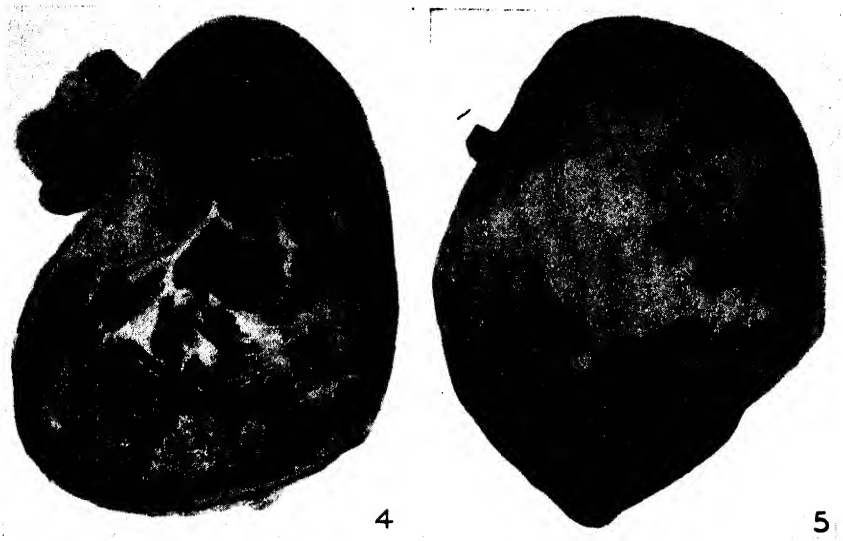


FIG. 4. *Celloidin section through maximum diameter of the sucker ventricle in diastole, to illustrate relation between muscle volume and cavity volume. ×7. Weight of fish, 465 gm. Beat rate, 21 per minute. The dark mass in the cavity is blood. A portion of the atrioventricular tissue can be seen attached to the ventricle.*

FIG. 5. *Celloidin section through the maximum diameter of the catfish ventricle in diastole, to illustrate the relation between muscle and cavity volume. ×7. Weight of fish, 471 gm. Beat rate, 21 per minute. The blood is not present in the main cavity but can be seen as dark areas scattered throughout the section.*

It follows that for equal beat rates of the heart, the amount of blood circulated in a given time varies in different fish. This would tend to cause differences in the oxygen transport in the various species. The minute output of the four species studied is such as to confer on the catfish an advantage over the remaining species in that it circulates blood about one and one-half times as fast as the carp, almost twice as fast as the bowfin, and about two and one-half times as fast as the sucker, under the experimental conditions. A similar relation may exist under natural conditions.

The carbon dioxide sensitivity of the bloods of the four species studied by Black was such as to confer on the sucker a large advantage over the catfish in the unloading of oxygen to the tissues, and hence by virtue of this property of the blood, this fish should be able to transport more oxygen than the catfish for equal volumes of blood circulated. When the minute output and the carbon dioxide sensitivity of the blood are compared (Table I), they are seen to vary inversely. In the fish studied in Florida, a similar situation was found. In each species the respiratory tolerance to carbon dioxide was studied instead of the carbon dioxide sensitivity of the blood. Those fish that were tolerant to carbon dioxide (four species) had a larger cardiac output than others that were sensitive to carbon dioxide (two species). The blood

pressure (bulbus arteriosus) was also found to vary in the Florida species, those with a large stroke output possessing a lower blood pressure than others with a small output.

It thus appears that differences in both the chemical and mechanical transport of oxygen have to be considered in order to explain similarities or dissimilarities in the oxygen consumption by different species. That these two factors may compensate for each other to some extent has been indicated above. Since species with blood of high sensitivity to carbon dioxide do not necessarily consume oxygen at a faster rate than those with blood of low sensitivity to carbon dioxide (2), the circulation may be paramount in compensating for differences in transport of oxygen by virtue of the carbon dioxide effect. However, other differences in the chemical properties of the bloods of these species may compensate for differences in carbon dioxide effect. For example, the inverse relation between the carbon dioxide effect on blood and the affinity of the same blood for oxygen was demonstrated in these species by Black (1) and is shown in Table I.

The relation of the bloods of the four species to the environments in which they lived was pointed out by Black. Blood with high sensitivity to carbon dioxide and low affinity for oxygen was associated with cold water habit (common sucker), and blood with low sensitivity and high affinity for oxygen was associated with warm water habit (catfish). Thus the former species is found in the deep water in Algonquin Park lakes during summer while the latter is found in shallow surface water. Similarly, small cardiac output may be associated with cold water habit and large output with warm water habit in these lakes.

Acknowledgments

Sincere thanks are due Dr. F. E. J. Fry who suggested the problem, and who was most liberal with his time and guidance during the course of the work.

The writer would also like to express thanks to Professors E. Horne Craigie and W. J. K. Harkness for advice, and to Dr. K. C. Fisher for use of equipment in the laboratory of experimental biology. The writer is grateful to Dr. Laurence Irving of Swarthmore College, Pa., for criticism of the manuscript.

References

1. Black, E. C. The transport of oxygen by the blood of freshwater fish. Biol. Bull. 79 : 215-229. 1940.
2. Black, E. C., Fry, F. E. J., and Scott, W. J. Maximum rates of oxygen transport for certain freshwater fish. Anat. Record, 75 (Suppl.) : 80. 1939.
3. Hart, J. S. The circulation and respiratory tolerance of some Florida freshwater fish. Proc. Florida Acad. Sci. (In press).
4. Krogh, A. and Leitch, I. The respiratory function of the blood in fishes. J. Physiol. 52 : 288-300. 1919.
5. Mathison, G. C. The effects of potassium salts upon the circulation and their action on plain muscle. J. Physiol. 42 : 471-494. 1911.
6. Root, R. W. The respiratory function of the blood of marine fishes. Biol. Bull. 61 : 427-456. 1931.
7. Willmer, E. N. Some observations on the respiration of certain tropical freshwater fishes. J. Exptl. Biol. 11 : 283-306. 1934.

CANADIAN WILTSHIRE BACON

XXIII. THE EFFECT OF CONCENTRATION OF CURING SALTS ON COLOUR AND COLOUR STABILITY[1]

By A. H. Woodcock[2] and W. Harold White[3]

Abstract

Small cuts of pork back were cured in pickles containing six concentrations each of chloride, nitrate, and nitrite varying from zero to above the concentrations normally used commercially. Quantitative colour measurements were made by an improved three-colour method on the internal surfaces of the lean meat. Salt contents were determined for each sample.

Increase of chloride concentration retarded methaemoglobin formation as indicated by a relative increase in the green and corresponding decrease in the red colour components. Variations in the nitrate content of the meat appeared to have no significant effect on the colour. The presence of nitrite in the meat caused the appearance of an absorption band in the region of 490 mμ, and a retardation of methaemoglobin formation as indicated by the red and green components of the initial colour and also by colour stability. The absorption band at 490 mμ is considered to be due to nitrosohaemoglobin. The presence of approximately 50 p.p.m. of nitrite in the meat appears to have been sufficient for complete reaction.

Introduction

The characteristic colour of bacon is attributed primarily to nitroso compounds formed by the reaction of nitrite with blood and muscle pigments of the haemoglobin type (1, 3, 5, 8, 10). Chloride and nitrate, the other two salts normally used in curing Wiltshire bacon, are reported to have some effect also on the resulting colour (2, 4, 7, 9).

Relations between the chloride, nitrate, and nitrite contents of commercially cured bacon and quantitative measurements of the colour by means of a photoelectric comparator provided with three filters have been reported previously (13, 14). However, the small differences observed in the contents of the curing salts made definite estimation of their effect on colour difficult (6). Moreover, a recent study has shown that a more suitable evaluation of the colour of bacon may be obtained by the use of a photoelectric colour comparator employing a new principle in the separation of the three colour bands (15). The present paper describes an investigation in which quantitative measurements by the improved method were made of the colour quality and stability of laboratory-cured bacon containing widely varying amounts of chloride, nitrate, and nitrite.

[1] *Manuscript received January 21, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 87 of the Canadian Committee on Food Preservation, and as N.R.C. No. 1107.

[2] *Biophysicist, Food Preservation Investigations.*

[3] *Biochemist, Food Preservation Investigations.*

Material and Methods

The material consisted of one full-length, rib-in, export pork back taken from each of 18 hogs specially selected as to uniformity of size and quality. Three pieces approximately 5 in. in length were cut from the front end of each back. One sample was allotted at random to each of the various curing treatments.

The investigation was divided into two parts in order to obtain a sufficient range of conditions without the use of an impractically large number of samples. The first part contained all possible combinations of 0, 15, and 30% sodium chloride, 0, 1, and 5% sodium nitrate, and 0, 0.1, and 0.5% sodium nitrite; and the second part, all possible combinations of 5, 10, and 20% sodium chloride, 0.5, 3.0, and 10% sodium nitrate, and 0.05, 0.2, and 1.0% sodium nitrite. Each sample was cured for 70 hr. at 4.4° C. (40° F.), after which it was wiped, drained for 24 hr., wrapped in waxed paper, and matured for nine days at 7.1° C. (45° F.).

The chloride, nitrate, and nitrite contents of each sample were determined by methods previously described (12). The colour of the freshly cut surface of the lean meat was measured with a photoelectric comparator (15). Brightness was determined as the total amount of light scattered by the sample as compared with a standard white surface. Colour quality was estimated as the percentage distribution of the total light scattered by the sample in the blue, green, and red bands, namely, 464 to 525 mμ, 525 to 600 mμ, and above 600 mμ, respectively.

In order to obtain an estimate of colour stability, the sample, after the initial measurement, was exposed to the atmosphere for 70 hr. at 7.1° C. and 95% relative humidity, and its colour remeasured.

Results and Discussion

Initial Colour

Mean values for colour and for the concentrations of sodium chloride, nitrate, and nitrite in the bacon at each concentration of the curing pickle are shown in Table I. An increase in the concentration of sodium chloride was associated with an increase in the red scatter, and a decrease in both the green scatter and total brightness. The blue intensity was relatively unaffected by change in the chloride concentration.

Statistical analyses were used to assess the significance of the observed changes. By means of partial correlation coefficients it was possible to ascertain the independent effect of each salt on the colour of pork. It will be seen from Table II that the changes with chloride concentration in the red and green intensities and in total brightness were statistically significant.

The increase in red and corresponding decrease in green intensity are presumably due to a retardation of methaemoglobin formation as the concentration of chloride is increased. It has been reported that chloride, when present in large amounts, markedly accelerates the formation of methaemo-

TABLE I

MEAN VALUES FOR THE COLOUR AND CONCENTRATION OF CURING SALTS IN BACON

Curing salt	Concentration of curing salts		Scatter, %			Brightness, %
	Pickle	Bacon	Blue	Green	Red	
Sodium chloride	0 %	0 %	34.8	33.3	31.9	18.2
	5	1.29	35.2	33.4	31.4	16.0
	10	2.12	34.5	33.6	31.9	14.6
	15	2.98	34.5	33.0	32.5	15.7
	20	3.80	34.6	32.9	32.5	13.5
	30	5.14	34.4	32.5	33.1	14.6
Sodium nitrate	0 %	0 %	35.0	32.4	32.6	16.3
	0.5	0.04	34.5	33.2	32.3	14.6
	1.0	0.06	34.3	33.2	32.5	16.7
	2.0	0.29	35.0	33.3	31.6	15.0
	5.0	0.52	34.5	33.2	32.3	15.6
	10.0	1.45	34.9	33.1	32.0	14.5
Sodium nitrite	0 p.p.m.	4 p.p.m.	36.0	32.4	31.6	16.6
	500	59	34.8	33.3	31.9	15.7
	1000	96	33.7	33.2	33.1	15.4
	2000	202	34.8	32.9	32.3	14.2
	5000	407	34.1	33.2	32.7	16.6
	10000	1046	34.9	33.4	31.7	14.2

TABLE II

PARTIAL CORRELATION COEFFICIENTS BETWEEN COLOUR AND THE CONCENTRATION OF CURING SALTS IN BACON

Quantities correlated	Scatter			Brightness
	Blue	Green	Red	
Chloride independent of nitrate and log nitrite	0.04	−0.34*	0.47**	−0.59**
Nitrate independent of chloride and log nitrite	0.18	0.02	−0.14	−0.21
Log nitrite independent of chloride and nitrate	−0.48**	0.19	0.31*	−0.34*

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

globin (4). The results of the present investigation suggest that this conclusion is not valid for such concentrations of sodium chloride as are normally present in Wiltshire bacon. The changes in total brightness show that the meat becomes darker in colour with increase in chloride concentration.

Nitrate had little effect on the colour of bacon (Tables I and II). While the total brightness in general decreased with increase in the concentration of nitrate, the differences were not statistically significant.

An increase in the concentration of nitrite in the meat caused a reduction in the blue intensity and in brightness, and an increase in the red intensity (Table I). The amount of light scattered in the green was relatively unchanged. Partial correlation coefficients showed that the observed changes in the blue and red scatter, and in brightness were statistically significant (Table II). Since the relation between nitrite concentration and colour scatter is obviously not linear (Table I), logarithms of the nitrite content were employed in these computations as a closer approximation to the actual function.

The major portion of the observed changes occurred in the blue region and for concentrations of nitrite in the meat of up to approximately 50 p.p.m. If the colour changes are attributed to a chemical reaction of nitrite with the meat pigment, then the indications are that the reaction is complete when an excess of approximately 50 p.p.m. of nitrite is present. The compound formed has an absorption band in the blue region (464 to 525 mμ), in agreement with previous investigations (11, 15). In addition, the compound is more resistant to methaemoglobin formation than the normal meat pigments since the red scatter is increased by the addition of nitrite.

There is some indication that nitrite, like chloride, reduced the brightness of the meat. Since the values are variable, however, it is unknown whether this reduction is due to the chemical reaction of the nitrite with the meat pigments, causing a reduction of the blue scatter, or to a more general reduction in brightness comparable with the effect of chloride.

Colour Stability

The changes on exposure to air in colour quality and brightness were generally small and irregular. An analysis of variance of these changes according to pickle composition revealed that nitrite was the most important of the three curing salts in contributing to colour stability (Table III). It appears that the change in colour is due to methaemoglobin formation, and that the reaction product of nitrite is more resistant to such a change than the normal meat pigments. The observed decrease in brightness on exposure bore no statistically significant relation to the various cures.

TABLE III

MEAN VALUES FOR THE EFFECT OF NITRITE ON THE COLOUR STABILITY OF BACON

Nitrite in curing pickle	Scatter, %			Brightness, %
	Blue	Green	Red	
Present	0.0	+2.5	−2.5	−0.28
Not present	−0.7	+4.3	−3.6	−0.26

Acknowledgments

The authors wish to thank Dr. J. W. Hopkins, Statistician, National Research Laboratories, for his advice on the statistical computations, and Messrs. H. Tessier, M. L. McMahon, and D. A. Wright, Laboratory Assistants, for their technical assistance.

References

1. Anon. Food Manuf. 12 : 230-231. 1937.
2. Brooks, J. Proc. Roy. Soc. (London) B, 109 : 35-50. 1931.
3. Brooks, J. Proc. Roy. Soc. (London) B, 123 : 368-382. 1937.
4. Brooks, J. Food Research, 3 : 75-78. 1938.
5. Brooks, J., Haines, R. B., Moran, T., and Pace, J. Dept. Sci. Ind. Research (Brit.), Spec. Rept. Food Investigation Board, No. 49. 1940.
6. Cook, W. H. and White, W. H. Can. J. Research, D, 18 : 135-148. 1940.
7. Hoagland, R. U.S. Dept. Agr., Ann. Rept. Bur. Anim. Ind., 1908, pp. 301-314. 1910.
8. Jones, O. Food, 6 : 238-240. 1937.
9. Sidwell, A. E., Jr., Munch, R. H., Barron, E. S. G., and Hogness, T. R. J. Biol. Chem. 123 : 335-350. 1938.
10. Urbain, W. M. and Jensen, L. B. Abstract. Food Manuf. 12 : 377. 1937.
11. Urbain, W. M. and Jensen, L. B. Food Research, 5 : 593-606. 1940.
12. White, W. H. Can. J. Research, D, 17 : 125-136. 1939.
13. Winkler, C. A., Hopkins, J. W., and Rooke, E. A. Can. J. Research, D, 18 : 225-232. 1940.
14. Winkler, C. A., Hopkins, J. W., and Thistle, M. W. Can. J. Research, D, 18 : 217-224. 1940.
15. Woodcock, A. H. Can. J. Research, D, 21 : 90-97. 1943.

COLOUR OF MEAT

IV. MEASUREMENT OF THE COLOUR OF BACON[1]

By A. H. Woodcock[2]

Abstract

Spectrophotometric measurements were used to study the reflection spectrum of pork and bacon in detail. A new method of colour measurement using nine filters was developed and tested experimentally on 100 samples of bacon treated in various ways.

It was found that three distinct changes could occur in the colour quality of bacon which were related to known physical or chemical changes. A loss of scatter in the blue region was associated with the reaction between nitrite and the haemoglobin pigments. An increase of scatter in the green with a corresponding decrease of scatter in the red was interpreted as loss of haemoglobin and a corresponding increase of methaemoglobin pigment.

These changes normally occurring in the colour of bacon can be assessed with three filters which absorb light below wave lengths of 460, 520, and 595 mμ, thus simplifying the method without loss of efficiency.

The estimation of the total scatter of the bacon is made by an independent measurement.

Introduction

A comparator for estimating objectively the colour of meat or other similar materials has been described in earlier papers of this series (6, 8). The instrument was based on the standard tricolour method of defining colour in the blue, green, and red spectral regions. These were isolated by filters. The values for light scattered in each spectral region were expressed as a percentage of the scatter from a standard white surface in the same region. The sum of the scatters by each component yielded a relative estimate of brightness.

Theoretically this comparator was capable of estimating two attributes of colour, chroma or colour quality and brightness. Practical experience (3, 4, 7, 9, 10, 11, 12, 13) demonstrated that the instrument was entirely satisfactory for studying changes that affected brightness primarily (3, 4, 7). It was less satisfactory for estimating colour quality since the scatter values for meat in the three broad regions of the visible spectrum studied were generally correlated (10). In addition it was found difficult to demonstrate that independent fluctuations significantly exceeded the experimental and sampling errors.

The present investigation was undertaken to determine the wave bands required to specify the colour of bacon, their width, number, and position in the visible spectrum, and the method most suitable for measuring these bands and interpreting the results.

1. *Manuscript received January 21, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 88 of the Canadian Committee on Food Preservation and as N.R.C. No. 1108.

2. *Biophysicist, Food Investigations.*

Spectrophotometric Examination

The first phase of the investigation consisted of a detailed spectrophotometric examination of the light scattered from the surface of fresh pork and of smoked and unsmoked bacon, to determine the width and position of those regions of the visible spectrum in which colour changes occur. The spectrophotometer, equipped with a Hilger constant deviation spectrometer as monochromator, was used with a resolving power of 6 mμ in the region of 589 mμ. Observations were made in the visible spectrum at 5 mμ intervals from 425 mμ in the violet to 680 mμ in the red. All measurements were expressed as a percentage of the light scattered in the same region by a standard white surface of magnesium carbonate.

Typical curves for fresh pork and for unsmoked and smoked bacon, shown in Fig. 1, serve to demonstrate the principal features of the colour. The

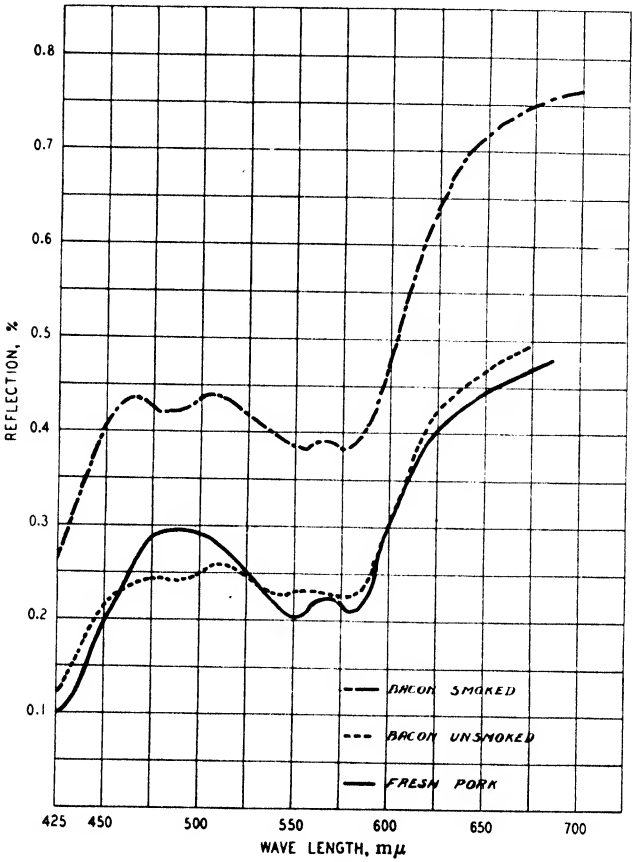


FIG. 1. *Typical spectrophotometer curves for fresh pork and unsmoked and smoked bacon.*

minima at 545 mμ and at 575 mμ are common to all three curves and in addition were found to be present in diluted pigs' blood. They correspond to those reported by Sidwell and others (5) for oxyhaemoglobin, and also to those reported by Brooks (1) for nitrosohaemoglobin. Consequently they were interpreted as absorption bands characteristic of both oxy- and nitroso-haemoglobin. Bacon in contrast to pork has an absorption band centred near 490 mμ which is considered to be characteristic of nitrosohaemoglobin (14).

The effect of smoking was to increase the amount of light scattered without changing the contour of the curve to any great extent. Hence curing affected the colour quality of the meat, while smoking, on the other hand, changed the brightness.

Spectrophotometric measurements by this method were not suited for extensive work on inherently variable biological materials where a large number of observations are necessary to reach valid conclusions. The resolving power of the spectrophotometer was unnecessarily high since observed changes in the scatter were due to absorption bands of the order of 25 mμ in width. Further it is doubtful if, under normal conditions, the eye could detect a differential change in the colour of meat between two wave lengths of less than this amount.

Examination by Filter Methods

Three Filter Method

The spectrophotometric transmission curves of the blue, green, and red filters used in the three colour comparator are shown in Fig. 2. From these curves it is evident that the comparator was relatively insensitive to changes in scatter in the regions near 485 mμ and 575 mμ, the bands indicated by spectrophotometric measurements to be of greatest importance.

It is a well known property of filters that the lower wave length limit is much sharper than the upper. This is illustrated by the curves in Fig. 2 where the green filter cuts off quite sharply on the short wave length side at 500 mμ but tapers gradually from 540 to 580 mμ on the long wave length side. Such filters are unsuitable for isolating bands of the order of 50 mμ in the visible spectrum. More selective filter combinations are available but are so dense that they transmit an insufficient amount of light to operate the photocell.

Nine Filter Method

From the above considerations the requirements of a comparator yielding more information on the colour could be estimated. Such requirements were that the width of any colour band be not more than 50 mμ, all portions of the spectrum be emphasized as equally as possible, the method be reasonably rapid so that large numbers of samples could be studied, and if possible that the measurements be made in such a manner that the results could be easily interpreted in terms of colour quality and brightness.

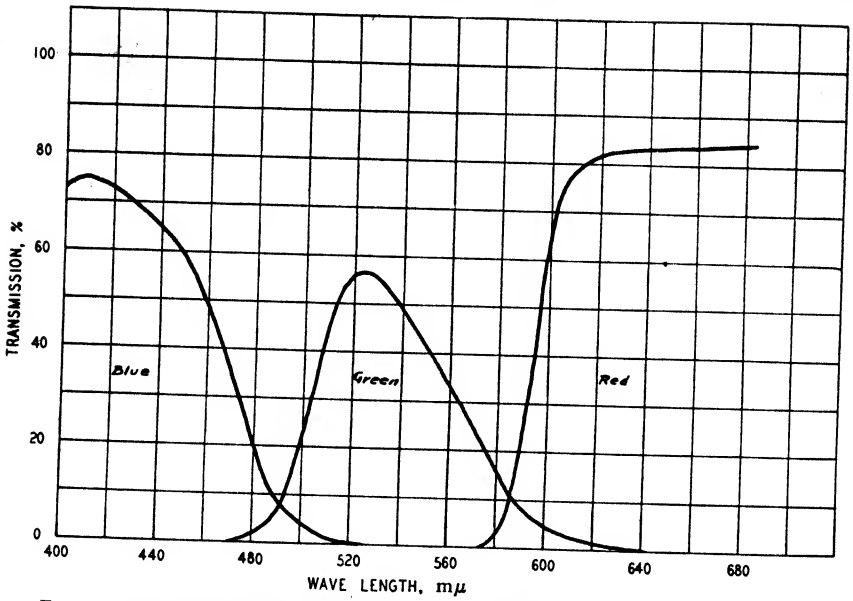


FIG. 2. *Spectrophotometric transmission curves of filters used in the three colour method.*

A new method for using light filters was chosen to fulfil these requirements. Nine filters of the type that transmits only light above certain characteristic wave lengths were arbitrarily selected to cover the entire visible spectrum at approximately equal intervals. From the transmission curves of these filters, as shown in Fig. 3, it will be seen that all had sharp cut-offs distributed over the entire visible spectrum. To measure brightness the scatter from the sample was compared with and expressed as a percentage of the scatter from a standard white surface using the filter transmitting all visible light.

Colour quality was measured by successively cutting off portions of the light scattered by the sample. By subtracting adjacent observations eight differences were obtained which, together with the transmission through the last dark red filter, made up 100% of the scattered light. The bands so formed are represented in Fig. 4. The scatter in each band was thus expressed as the percent of total scatter.

This satisfied the requirements mentioned above. The bands were all less than 45 mμ in width and were such that equal sensitivity was obtained over the whole visible spectrum. The colour quality was measured independently of the brightness.

For these experimental tests a temporary apparatus was used in which one of the three colour comparators was modified to permit the use of nine filters and a second comparator employed as a compensating source to eliminate the fluctuations of light intensity caused by variations in line voltage. Such compensation was found necessary since measurement of differences in

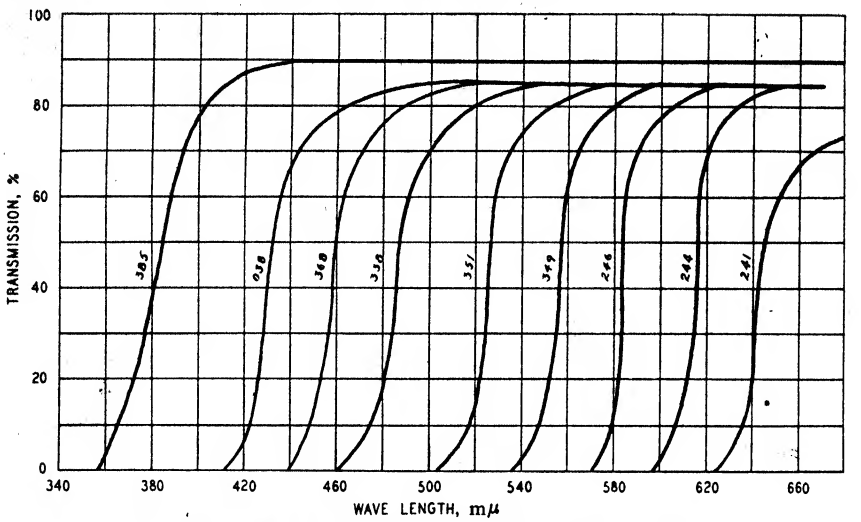


FIG. 3. *Spectrophotometric transmission curves of filters used in the nine colour method.*

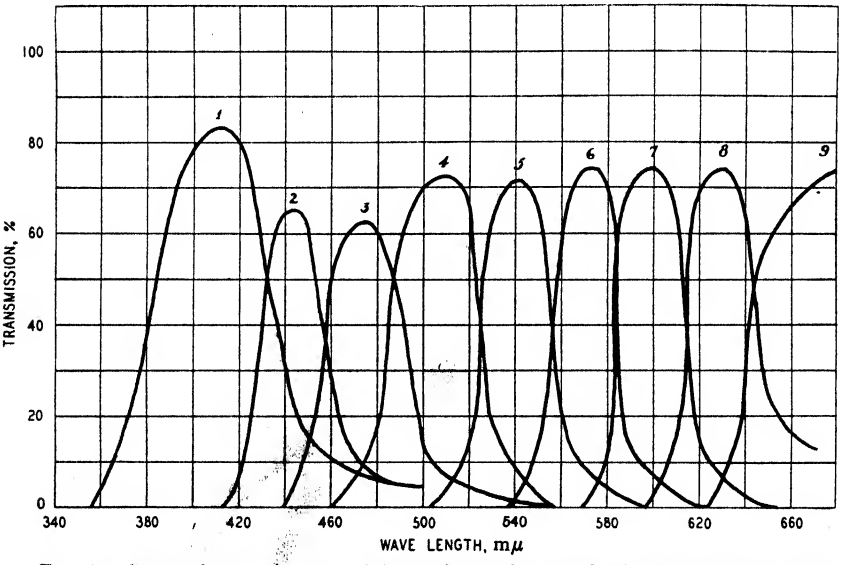


FIG. 4. *Spectrophotometric curves of the resultant colour bands obtained when using the nine colour method.*

scatter amplified the instrumental errors. The compensating circuit consisted of a fixed resistor placed in parallel with the photocell of the nine colour comparator and a calibrated variable resistor likewise in parallel with the compensating photocell. The voltages developed across these resistors were balanced using a galvanometer as a null instrument.

This type of apparatus, while it proved satisfactory for test purposes, could be greatly improved. The use of vacuum type photocells in a modern balanced electronic circuit, and a calibrated diaphragm to equalize the intensity of the light on the photocells would offer considerable improvement.

Comparison of Three and Nine Filter Methods

The relative ability of the three and nine colour methods to distinguish between colour quality and brightness was studied on two groups of samples. The first group, selected to illustrate changes due to methaemoglobin formation, consisted of 50 samples of smoked bacon selected at random from other studies in which storage conditions only were varied. The second group, considered to illustrate both methaemoglobin and nitrosohaemoglobin formation, consisted of 50 samples of unsmoked bacon, 27 of which were known to have variations in colour due to differences in curing pickles, the other 23 being chosen at random from a large number varying in either cure or storage history.

The experimental error for each band was determined by making duplicate measurements. The samples were removed after each measurement so that this error would include variations in the instrument and in the orientation of the bacon in the sample holder.

Results

Correlation coefficients were computed between the light scattered in each colour band by the three filter method, and the brightness measured by the new method (Table I). It may be seen that the coefficients are very high, indicating that approximately 90% of the variance encountered with the three colour instrument is due to brightness, leaving the remaining 10% to account for changes in colour quality, independent variations, and error. This supports the statement that the three filter method indicated mainly changes in brightness.

TABLE I

CORRELATION OF BRIGHTNESS AS MEASURED BY THE NINE COLOUR METHOD WITH COLOUR AS DETERMINED BY THE THREE COLOUR METHOD

Quantities correlated	Smoked bacon		Unsmoked bacon	
	D.f.	r	D.f.	r
Brightness × blue	48	+ 0.96	48	+ 0.94
Brightness × green	48	+ 0.96	48	+ 0.96
Brightness × red	48	+ 0.93	48	+ 0.93

Data obtained by the nine filter method were reduced by computing the standard deviations and standard errors of each band and the correlation coefficients between adjacent bands as shown in Table II. It may be seen that in the majority of the bands the standard deviation or variability of the

material measured exceeded the instrumental errors, indicating that the method has adequate precision.

TABLE II

COLOUR QUALITY OF BACON—INSTRUMENTAL ERROR, STANDARD DEVIATION, AND CORRELATION BETWEEN ADJACENT BANDS AS MEASURED BY THE NINE COLOUR METHOD

Band	Mean square error (19 deg. freedom)	Smoked bacon				Unsmoked bacon			
		Standard deviation (49 deg. freedom)	Bands correlated	D.f.	r	Standard deviation (49 deg. freedom)	Bands correlated	D.f.	r
1	7.8	26.5**	1 and 2	48	0.001	15.9**	1 and 2	48	−0.03
2	5.3	31.9**	2 and 3	48	0.01	29.7**	2 and 3	48	−0.05
3	5.4	8.6*	3 and 4	48	0.01	17.2**	3 and 4	48	0.36*
4	5.0	15.7**	4 and 5	48	0.69**	39.6**	4 and 5	48	0.18
5	1.5	15.6**	5 and 6	48	0.87**	30.2**	5 and 6	48	0.77**
6	2.4	74.0**	6 and 7	48	−0.63**	101.3**	6 and 7	48	−0.74**
7	1.5	16.6**	7 and 8	48	0.84**	33.2**	7 and 8	48	0.81**
8	3.1	31.6**	8 and 9	48	0.93**	41.9**	8 and 9	48	0.70**
9	1.8	33.2**				56.2			

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

In assessing the significance of the data obtained for the light scattered in the various bands it should be noted that a high correlation coefficient between adjacent colour bands indicates that the same change was observed in each. Correlations between Bands 1 and 2, 2 and 3, 3 and 4 for smoked bacon and between Bands 1 and 2, and 2 and 3 for unsmoked bacon were small, indicating that the variations, though exceeding the standard error, were independent. Since it seems unlikely that the filters arbitrarily selected should be such that they coincided with regular independent colour changes, it appears that inherent variations between samples were being detected.

High positive values of the correlation coefficients were obtained between Bands 4 and 5, 5 and 6, and between Bands 7 and 8, and 8 and 9; the negative coefficient between Bands 6 and 7 indicated that two related types of change occurred. Examination of the standard deviations showed that Bands 6 and 9 were most affected by the changes. The colour Bands 4, 5, and 6 can be identified by comparison with the spectrophotometric curves as being due to haemoglobin while Bands 7, 8, and 9 cover the region of methaemoglobin absorption (2). Thus both changes were caused by the oxidation of haemoglobin to methaemoglobin.

Examination of the results of colour measurements on unsmoked bacon indicated another type of change as well as that associated with methaemoglobin formation. This was indicated by a higher correlation between Bands 3 and 4 than between adjacent bands. Spectrophotometric studies, as mentioned previously, indicated that an absorption band occurred in this region and that it is formed as a result of curing.

Discussion

The results obtained above indicate that variations in the colour quality of bacon resulting from variations in curing or storage procedure can be assessed with three filters, thereby simplifying the method. The effect of other treatments on colour of bacon would have to be examined fully if it were suspected that they caused other types of colour change.

The nature of these changes led to the selection of the following three filters for the specification of colour. Since Bands 1 and 2 did not appear to be related to any specific change and are also in the extreme violet region of the spectrum where the eye is relatively insensitive they can be considered of no importance to visual colour. The band in the blue, related to curing practice, and extending from 460 mμ to 520 mμ, can be separated by two filters which cut off at these wave lengths. The remainder of the visible spectrum can then be divided into two regions, namely, those of haemoglobin and methaemoglobin absorption, by a third filter cutting off at 595 mμ.

In this way an instrument can be obtained to measure definite characteristics of colour quality and brightness independently, yet the method is as simple and rapid in operation as the original three filter method. This method may also be made suitable for colour measurement of paints, dyes, and textiles, etc. by using appropriate filters as determined by the above methods.

Acknowledgments

The author wishes to thank Dr. L. E. Howlett, Division of Physics and Electrical Engineering, National Research Laboratories, for the loan of the spectrometer, to express gratitude to Dr. W. H. Cook and Dr. J. W. Hopkins, Division of Applied Biology, National Research Laboratories, for their advice in the interpretation of the results, and to thank Mr. H. Tessier, Laboratory Assistant, for aid with the experimental measurements.

References

1. Brooks, J. Proc. Roy. Soc. (London) B, 123 : 368-382. 1937.
2. Brooks, J. Food Research, 3 : 75-78. 1938.
3. Cook, W. H. Can. J. Research, D, 19 : 85-95. 1941.
4. Cook, W. H. and White, W. H. Can. J. Research, D, 19 : 53-60. 1941.
5. Sidwell, A. E., Jr., Munch, R. H., Barron, E. S. G., and Hogness, T. R. J. Biol. Chem. 123 : 335-350. 1938.
6. Winkler, C. A. Can. J. Research, D, 17 : 1-7. 1939.
7. Winkler, C. A. Can. J. Research, D, 17 : 29-34. 1939.
8. Winkler, C. A., Cook, W. H., and Rooke, E. A. Can. J. Research, D, 18 : 435-441. 1940.
9. Winkler, C. A., Cook, W. H., Rooke, E. A., and Chadderton, A. E. Can. J. Research, D, 19 : 22-27. 1941.
10. Winkler, C. A. and Hopkins, J. W. Can. J. Research, D, 18 : 211-216. 1940.
11. Winkler, C. A. and Hopkins, J. W. Can. J. Research, D, 18 : 289-299. 1940.
12. Winkler, C. A., Hopkins, J. W., and Rooke, E. A. Can. J. Research, D, 18 : 225-232. 1940.
13. Winkler, C. A., Hopkins, J. W., and Thistle, M. W. Can. J. Research, D, 18 : 217-224. 1940.
14. Woodcock, A. H. and White, W. H. Can. J. Research, D, 21 : 85-89. 1943.

BEHAVIOUR AND NATURE OF THE FLUORESCING SUBSTANCES IN DRIED EGG POWDERS[1]

By J. A. Pearce[2]

Abstract

Fluorescence increased during storage in defatted, dried egg powders, and also in separately dried yolk and white. A portion of the fluorescent material was soluble in fat solvents and especially in alcohols. Ethanol may also destroy some of the fluorescing substance. The results indicate that fluorescent materials arise from changes in the protein fraction, that there is more than one fluorescing substance, and that some of these materials contribute to the increased fluorescence associated with decrease in quality. Indirect evidence indicates that proteose and peptone constituents are partly responsible for fluorescence, and that the nature of the deterioration is hydrolytic. A preliminary study was made of the effect of certain enzymes and micro-organisms on the formation of fluorescent materials. The work is being continued.

Introduction

The fluorescence of a potassium chloride extract of defatted dried egg powder was found to be closely correlated with the quality of the powder (3, 6). The urgent need of such a measurement delayed any detailed investigation of the fluorescent materials. The present investigation was concerned with the determination of the nature and behaviour of the fluorescing substances.

Materials and Procedure

The range of egg powders used in part of this investigation were kindly supplied by Canada Egg Products Ltd., Montreal.

The procedure used was essentially that previously described (3). However, in certain instances, fat and protein solvents other than chloroform, and 10% potassium chloride solution were used.

The results were recorded, as before, in photofluorometer units, but the variety of solutions used necessitated blank corrections to permit comparison of the results. Such corrections can be readily made since the scale of the Coleman photofluorometer is arranged to give a linear relation between scale reading and concentration of fluorescent material (cf. 3, Table I).

Source of Fluorescent Materials

An indication of the source of the fluorescent material was obtained by storing whole and chloroform defatted egg powder at 23.9° C. (75.0° F.). The results (Table I) show that fluorescence developed in egg powder regardless of the presence or absence of chloroform soluble components and indicated that changes in the protein fraction yield the fluorescing material.

[1] *Manuscript received February 22, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 89 of the Canadian Committee on Food Preservation and as N.R.C. No. 1112.

[2] *Biochemist.*

TABLE I

CHANGE IN FLUORESCENCE OF WHOLE AND CHLOROFORM DEFATTED EGG POWDER AT 23.9° C.

Material	Fluorescence reading[1] after storage for		
	0	Two months	Four months
Whole egg powder	27.0	40.3	69.0
Defatted egg powder	27.1	39.8	65.0

[1] *No blank corrections.*

If these changes are occurring in the protein fraction they may be common to proteins present in either the white or the yolk or both. To investigate this, yolks of fresh eggs were separated from the white and a small portion of the centre of the yolks carefully removed with a pipette. White, yolk, and the remaining yolk membrane with its adhering white were vacuum-ice dried and stored at 23.9° C. Table II shows the fluorescence readings after two

TABLE II

CHANGE IN FLUORESCENCE OF DRIED EGG YOLK, WHITE, AND MIXED YOLK AND WHITE, AT 23.9° C

Material	Fluorescence reading[1] after storage for		
	0	One month	Two months
Yolk	10.0	15.0	20.4
White	10.1	20.5	32.2
Mixed yolk and white	14.8	16.5	23.5

[1] *No blank corrections.*

months' storage. Initially yolk and white had approximately the same fluorescence. After storage the fluorescence of the dried white greatly exceeded that of the yolk, but the development of fluorescence was common to both dried white and dried yolk. Since there is very little fat in dried egg white, this is further indication that fractions other than fat are involved in deterioration.

Effect of Fat Solvents

The differential effect of fat solvents on fluorescence readings (3) may result from the presence of differentially soluble fluorescent materials, or may be due to solution or destruction of the fluorescing substance. The effect of fat solvents was therefore reinvestigated, with a view to clarifying the point. The pronounced reduction in fluorescence reading resulting from the use of ethanol (3) was observed for other alcohols and for glacial acetic acid (Table III).

The effect of ethanol as a solvent was studied. For this purpose egg powders with a range of quality were extracted with chloroform, and then with three 50 ml. portions of 95% ethanol. The ethanol extract was made up to 250 ml. and the fluorescence measured.

TABLE III

EFFECT OF FAT SOLVENTS ON FLUORESCENCE READING

Solvent	Fluorescence reading[1]
Glacial acetic acid (washed subsequently with chloroform)	1.0
Ethanol (abs.)	5.0
Isobutanol (sat. with water)	5.6
Methanol	6.0
Ethanol (95%)	6.8
Isobutanol	16.0
Chloroform[2]	36.8
Petrol ether	42.0
Acetone	43.0
Benzene	44.0
Ethyl ether	50.2
Toluene	54.5

[1] *Readings corrected for solution blank in all tables unless noted.*

[2] *Standard method (3).*

The logarithm of the photofluorometer readings of the alcohol extract plotted against computed taster score are compared with other curves in Fig. 1. It is apparent that a large portion of the fluorescent material was soluble in 95% ethanol. This material, after evaporation of the alcohol *in vacuo* at room temperature and dispersion in water, continued to fluoresce, but to a smaller extent than in alcohol. The dried material obtained from alcohol extraction was a pale yellow, amorphous solid. Although the fluorescence of the alcohol extract increases with decreasing quality, the difference in slopes of the curves for water and alcohol extraction indicates that alcohol soluble fluorescent material contributes less to the fluorescence of poor quality powders than it does to powders of good quality.

The fluorescence readings of the residual powder, after defatting with chloroform and extraction with alcohol, compare favourably with values previously observed when defatting was done with 3 : 1 petrol-ether–absolute-alcohol and extraction with 10% potassium chloride solution (3).

If solubility in the fat solvent were the only factor causing differences in fluorescence reading, it would be expected that the addition of fluorescence readings for the powder after alcohol extraction (Curve 4) and the fluorescence reading of the alcohol extract materials in water (Curve 3) would approximately equal the fluorescence readings in water (Curve 1). This is not

the case; therefore, it might be concluded that in addition to alcohol soluble and insoluble fluorescing substances, there are fluorescent materials destroyed by alcohol. Thus, the presence of more than one fluorescing substance is indicated.

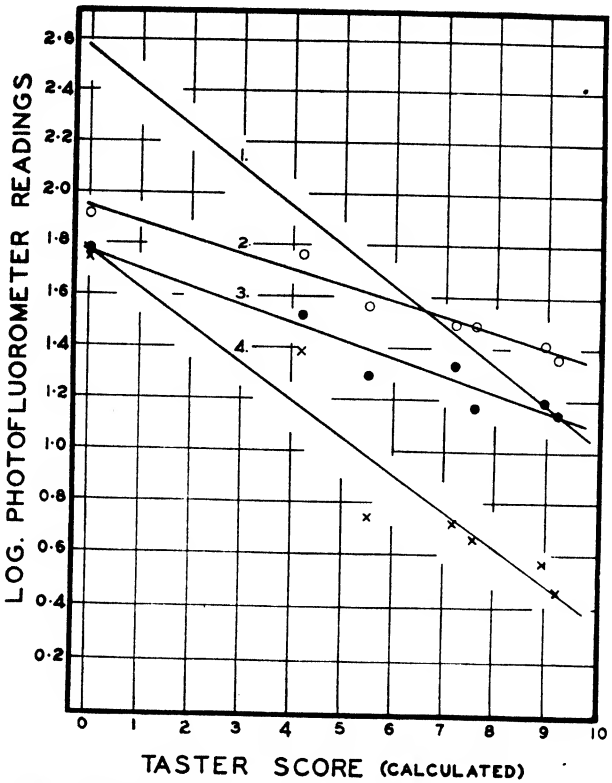


FIG. 1. *Effect of ethanol extraction on fluorescence reading. 1. Water extract of defatted powder. 2. Ethanol (95%) extract of defatted powder. 3. Water extract of alcohol soluble substance. 4. Potassium chloride (10%) extract of residue after ethanol extraction of defatted powder.*

Fluorescing Fractions

Lyophilic sols are known to fluoresce (2, p. 89). This phenomenon has been observed to occur in protein sols (5). Amino acids, the end products of protein decomposition, do not fluoresce (5). Foregoing work (3) indicates that the amount of fluorescence occurring in 10% potassium chloride extract prepared from fresh egg powder was small. Since fluorescence increases with decrease in powder quality, the highly fluorescent materials must, therefore, be intermediate products in protein decomposition.

Confirmation of this was sought by measuring the fluorescence of 1-gm. samples of commercial proteose–peptone mixtures (Difco), dispersed in 250 ml. 10% potassium chloride solution. These materials were found to have high

fluorescence values (Table IV). The values varied directly with free amino nitrogen content and pH and inversely with the diamino nitrogen and cystine content, as recorded by the manufacturer's analysis (1, p. 166). The full significance of these relations is not immediately evident, although the inverse relation with cystine content may point to breakdown of fluorescing substances to non-fluorescing amino acids as deterioration increases.

TABLE IV

FLUORESCENCE OF 0.4% PROTEOSE–PEPTONE MIXTURES IN 10% POTASSIUM CHLORIDE SOLUTION

Material	Fluorescence reading
Bacto–tryptone	216.7
Bacto–peptone	119.8
Proteose–peptone	83.4

Egg white is believed to contain about 80% ovalbumin, 10% ovomucoid, and 6.7% ovoglobulin, while egg yolk contains lecithin, lutein, cholesterol, vitellin, and livetin, the lecithin and vitellin probably existing in combination (4, p. 432). Of these, ovalbumin, ovomucoid, and livetin are soluble in water; ovalbumin, ovoglobulin, and lecithovitellin are soluble in dilute salt solutions. Proteoses and peptones are the protein decomposition products likely to be measured as they are soluble in water and dilute salt solutions; acid and alkali metaproteins and proteans are insoluble in these solutions.

Effect of Protein Solvents and Precipitants

The effect on the fluorescence reading of replacing 10% potassium chloride with 10% solutions of various salts and with protein precipitants (asbestos as a filter aid was omitted) is shown in Table V. Indications of the presence of any particular fluorescing protein are somewhat contradictory, again implying the presence of a number of fluorescing protein fractions. There are, also, indications of a lyotropic effect on the fluorescing substances; further evidence of their colloidal nature (7, p. 345).

Measurements were then made on a series of nine samples of dried egg powders with a range of quality to see if the fluorescing fractions were related to edibility. In addition the effect of filter aids on fluorescence readings was evaluated.

The mean fluorescence readings for each treatment together with an analysis of variance are given in Table VI. The very small fluorescence value in tannic acid solution was obtained with a sample of mixed milk and egg powder included in this series. Since all but this one reading were zero, these values were not included in the analysis of variance. The statistical analysis shows more fluorescing substances present in water extracts than in

TABLE V

EFFECT OF PROTEIN SOLVENTS AND PRECIPITANTS ON FLUORESCENCE READING

Solvent or precipitant	Fluorescence reading	Solvent or precipitant	Fluorescence reading
Sodium acetate	27.2	Sodium acetate	27.2
Sodium chloride	49.1	Lead acetate	8.6
Sodium nitrate	49.0		
Sodium sulphate	40.6	Sodium nitrate	49.0
Trisodium phosphate	23.4	Silver nitrate	0
		Cupric nitrate	0
Sodium chloride	49.1	Mercuric nitrate	0
Potassium chloride[1]	49.2		
Ammonium chloride	56.2	Picric acid (sat.)	0
Magnesium chloride	54.4	Tannic acid (0.5%)	0
Calcium chloride	40.0	Potassium ferrocyanide and acetic acid	0
Ferric chloride	0	Trichloroacetic acid	8.6
		Ammonium sulphate (sat.)	13.0
Ammonium sulphate	52.0	Magnesium sulphate (sat.)	23.0
Sodium sulphate	40.6		
Magnesium sulphate	40.2	Water	41.8

[1] *Standard method (omitting asbestos as filter aid).*

TABLE VI

EFFECT OF TREATMENT ON FLUORESCENCE READINGS OF EGG POWDERS OF DIFFERENT QUALITY

Treatment	Mean fluorescence reading	Treatment	Mean fluorescence reading
Water	70.5	Magnesium sulphate (sat.)	46.9
Water and asbestos	64.7	Lead acetate (0.5 *M*)	40.4
Water and Fuller's earth	51.6	Sodium chloride (sat.)	38.8
Potassium chloride (10%)	51.2	Ammonium sulphate (half sat.)	5.4
Potassium chloride (10%) and asbestos	54.3	Ammonium sulphate (sat.)	4.5
		Tannic acid (0.5%)	0.03

Necessary difference, 5% level = 5.2.

Analysis of variance

Variance attributable to:	D.f.	Mean square
Samples	8	41987**
Treatments	9	23190**
Samples × treatments	72	348**
Duplicate error	82	50

** *Exceeds 1% level of statistical significance.*

10% potassium chloride and saturated magnesium sulphate extracts while the latter contained more than 0.5 *M* lead acetate or saturated sodium chloride. Ammonium sulphate solutions extract very little and tannic acid solution extracts no fluorescent material. Fig. 2 shows representative curves of those extractions that showed statistical differences.

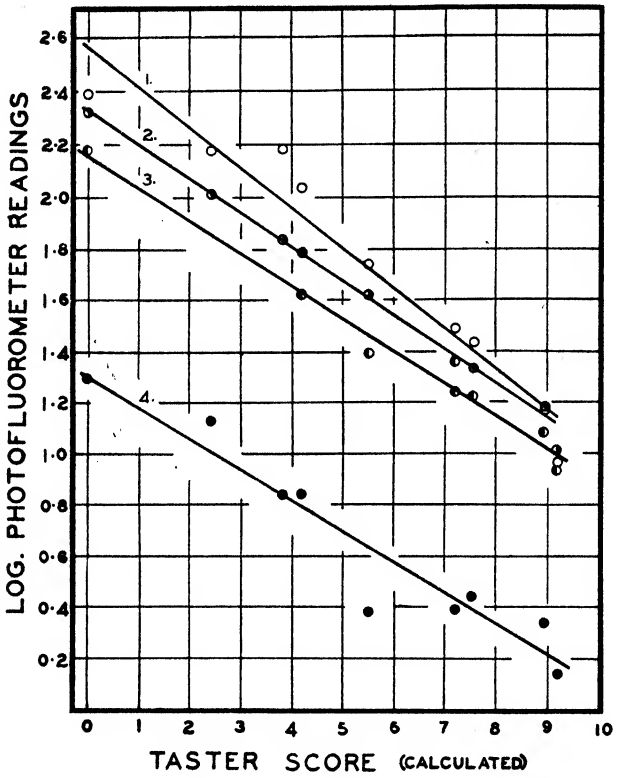


FIG. 2. *Effect of protein solvents and precipitants on fluorescence reading. 1. Water extract. 2. Potassium chloride (10%) extract. 3. Sodium chloride (saturated) extract. 4. Ammonium sulphate (half saturated) extract.*

The higher fluorescence values and greater range of readings obtained with water extraction are of no value in improving the method, because of extended filtering periods and poor agreement between duplicates. The use of sodium chloride, however, offers distinct practical advantages and is under investigation.

The results using filter aids showed that asbestos reduced fluorescence readings of water extracts, but made no significant change in readings when 10% potassium chloride solution was used. Fuller's earth did not remove the fluorescing substances completely from the water extracts, although it had been reported (5) to do so for other protein sols.

Effect of Heat on Formation of Fluorescing Substances

Water extracts of chloroform defatted powders were heated for 20 min. in a water-bath at 100° C. The presence of heat-coagulable fluorescent substances, or accelerated decomposition to non-fluorescing amino acids (5) should result in decreased fluorescence readings. On the other hand hydrolysis of non-fluorescing proteins to lower, fluorescent products should increase the readings. The latter case proved to be the more important, as shown by the increases noted in Table VII*a*. Partial fractionation of the heated extracts (Table VII*b*) supports the deduction that the nature of the change is hydrolytic. Further support is indicated by the effect of 1 *N* acids and bases (Table VIII) and by the work of others in these laboratories (8).

TABLE VII*a*

EFFECT OF HEATING WATER EXTRACTS OF DEFATTED, DRIED EGG POWDER

Treatment	Mean fluorescence reading
Water extract	88.4
Water extract, heated	116.1

Analysis of variance

Variance attributable to:	D.f.	Mean square
Samples	8	29343**
Treatments	1	6900**
Samples × treatments	8	544
Duplicate error	18	469

** *Exceeds 1% level of statistical significance.*

TABLE VII*b*

FRACTIONATION OF HEATED AND UNHEATED WATER EXTRACTS WITH AMMONIUM SULPHATE

Treatment	Mean fluorescence reading
(i) Unheated	
Ammonium sulphate (half saturated)	6.4
Ammonium sulphate (saturated)	5.3
(ii) Heated	
Ammonium sulphate (half saturated)	14.6
Ammonium sulphate (saturated)	12.2

Necessary difference, 5% level = 1.3.

Analysis of variance

Variance attributable to:	D.f.	Mean square
Samples	8	388**
Treatments	3	362**
Samples × treatments	24	12.3**
Duplicate error	36	3.46

** *Exceeds 1% level of statistical significance.*

TABLE VIII

EFFECT OF ACIDS AND BASES ON FLUORESCENCE READING

Material	Fluorescence reading in:		
	Water	1 *N* HCl	1 *N* NaOH[1]
Fresh egg powder	9.9	27.5	7.0
Old egg powder	35.0	27.5	11.5

[1] *Glass wool used for filtering.*

Effect of Enzymes and Bacteria on Formation of Fluorescing Substances

Hydrolysis as a feature of dried egg decomposition may be accelerated by the presence of enzymes. This was investigated by measuring the changes in fluorescence reading of 1% water sols of defatted egg powder containing 0.1 gm. of pepsin, papain, and trypsin per 100 ml. egg sol when incubated at 37° C. Increments in fluorescence readings greater than those occurring in the control were observed for papain and trypsin, as noted in Table IX. These measurements were difficult to make as enzymes in water have large fluorescence readings that decreased on incubation. It was impossible to determine whether or not the same decrease in fluorescence of enzymes occurred in the egg–enzyme mixtures.

TABLE IX

INCREMENTS IN FLUORESCENCE READINGS OF SOLUTIONS OF DEFATTED EGG POWDER IN WATER CONTAINING ENZYMES (pH = 6.9)

Treatment	Increment in fluorescence reading during incubation at 37.0° C.[1]			
	After 12 hr.		After 24 hr.	
Pepsin	−5.4	(+1.8)	4.6	(19.0)
Papain	14.0	(14.0)	18.2	(18.2)
Trypsin	6.4	(16.4)	26.4	(46.4)
Control	1.1		12.5	

[1] *Numbers in parentheses indicate values if decrement in fluorescence of enzyme solutions can be applied to enzyme–egg mixtures.*

No correlation between fluorescence reading and total bacterial count was apparent in previous work (6). However, a preliminary investigation, using defatted, dried egg in water as a medium for several strains of micro-organisms isolated from whole egg powder, showed that growth of certain of these organisms caused an increase in fluorescence reading*.

**Measurements made in the course of work (unpublished) by Mr. C. O. Fulton of these laboratories.*

Since there is a need to improve the keeping quality of dried whole egg owder, possible methods of retarding protein deterioration are under ıvestigation.

References

DIFCO MANUAL of dehydrated culture media and reagents. 6th ed. rev. Difco Laboratories Inc., Detroit. 1939.

GORTNER, R. A. Outlines of biochemistry. 2nd ed. John Wiley and Sons, Inc., New York. 1938.

PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20 : 276-282. 1942.

PLIMMER, R. H. A. Practical organic and bio-chemistry. New ed. Longmans, Green and Co., Ltd., London. 1926.

REEDER, W. and NELSON, V. E. Proc. Soc. Exptl. Biol. Med. 45 : 792-794. 1940.

THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.

THOMAS, A. W. Colloid chemistry. McGraw-Hill Book Company, Inc., New York. 1934.

WHITE, W. H. and THISTLE, M. W. (In preparation).

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. MAY, 1943 NUMBER 5

SOME NATIVE SAWFLIES OF THE GENUS *NEODIPRION* ATTACKING PINES IN EASTERN CANADA[1]

BY C. E. ATWOOD[2] AND O. PECK[3]

Abstract

The genus *Neodiprion* has become more and more important in recent years because of the variety of species found on our native conifers and the increasing frequency of outbreaks caused by these insects. It is very difficult to distinguish between the species by means of adult characters only, so a study of different stages of the life history has been made. In this way it has been found possible to delimit most of the species with a fair degree of certainty.

Keys are given for the determination of adult females of 11 and of larvae of 10 species attacking pines in Eastern Canada. Two of the species are described as new. The larvae of two species appearing in the first key and the adults of one species from the second are as yet unknown.

Introduction

During the past 15 years the sawflies of the genus *Neodiprion* have attracted an increasing amount of attention from forest entomologists in Canada. This has been due not only to the more common occurrence of damage to plantations and forests but also to the variety of species that has been found on our native conifers. This paper is an attempt to place on record a portion of the very considerable body of data relating to Eastern Canadian species that has now been accumulated, especially during the last four years, and to make possible the identification of the species that are likely to be encountered by forest entomologists and students of Hymenoptera.

Most of the field work on which this study is based was carried out at the entomological field station at Laniel, Que., where many of the species concerned have been reared from egg to adult. This was supplemented by further studies at Ottawa, where a certain amount of temperature control was possible and where methods have been devised for the rearing in incubators of most of the species concerned, although *N. pinetum* Nort. still presents difficulties. As a result of this work, good series of adults are available in most species, some series comprising several generations of descendants from a single pair. The field work has shown that the various species differ from each other in certain details of their life history, and that these differences can be used in separating them. Characteristics that have been used are

[1] *Manuscript received February 2, 1943.*

Contribution No. 2163 from the Division of Entomology, Science Service, Department of Agriculture, Ottawa.

[2] *Assistant Entomologist, Forest Insect Unit, Division of Entomology, Science Service.*

[3] *Assistant Entomologist, Systematic Entomology Unit, Science Service.*

larval coloration, spacing of eggs on needles, colour of cocoon, season in which adults emerge from cocoons, stage in which the winter is spent, gregarious or non-gregarious feeding habits of larvae. By a consideration of these characters it has been possible to segregate certain groups of individuals as representatives of true species; that is, it has been found that such groups behave as biological units. Once this has been done, it is possible to deal with adult material on a rational basis. In difficult and closely related groups such as these representatives of *Neodiprion*, it is practically impossible in some species to determine, from adult characters alone, the line between interspecific and intraspecific variation. However, when the range of variation has been examined in a series of adults that have been reared from larvae of a definite type and whose life history distinguishes them sharply from related groups of similar status, the taxonomic value of such variations can be evaluated. This "semi-genetical" method of attack has supplied the foundation for the present work and it is felt by the authors that many other problems in the taxonomy of forest insects can be solved only by similar methods.

The insects considered below include all the native species of this genus known to attack white pine (*Pinus Strobus* L.), red pine (*P. resinosa* Ait.), and jack pine (*P. Banksiana* Lamb.) in temperate Canada, east of the Manitoba boundary. No data are available for pitch pine (*P. rigida* Mill.) which is native to Canada only in very small areas along the extreme southern borders of eastern Ontario and New Brunswick (4). The species attacking western pines and those on other conifers have not yet been sufficiently investigated to yield material for a comprehensive treatment.

The writers have not included *Neodiprion abietis* Harr. among the pine feeders, although it has been recorded as one by some authors, including Bird (2) and Ruggles (23). They have been unable to find *abietis* on any pines in this region and all attempts to rear it from the egg on pines have failed. The female of this species, as understood by the writers, is readily distinguished (*a*) by the lancet of the ovipositor being unusually thickly haired, this area extending to the dorsoposterior margin and (*b*) by the scopal pad being shortly ovate.

With respect to hosts, it should be noted that the markings of larvae may vary within a species because of difference in host, as in *N. lecontei* (Fitch), but these variations are seldom, if ever, such as to place the specimen in the wrong section of the key, especially if larval descriptions are also consulted.

Identification of Adult Females

By O. Peck

In 1918 Rohwer (18) erected the genus *Neodiprion** for the reception of numerous North American species and one European species (*sertifer* Geoff.), that had been referred previously to *Lophyrus* Latr. and *Diprion* Schr.; he

* *Diprion, Zadiprion, and Neodiprion are words in the masculine gender, being derived from* πρι'ων *(prion), masculine, a saw.*

recognized two subgenera, *Neodiprion* (type *Lophyrus lecontei* Fitch) and *Zadiprion* (type *Diprion grandis* Roh.). Ross (22), in his synopsis of the nearctic genera of sawflies, considered these two groups as generically distinct, a course in which he was followed by Benson (1) in his synopsis of the diprionid genera of the world.

The genus *Neodiprion*, so limited, is readily separated from other nearctic genera of the Diprionidae by means of the characters tabulated by Ross (22). Unfortunately, the genotype (*lecontei*) falls in the first part of Couplet 1 of Benson's (1) key and hence does not run to *Neodiprion*. The character employed is the form of the anterior angle of the scutellum which is stated to be either much more than, or not greater than, a right angle. In *lecontei* the angle is about 115° (12, Plate 88; 22, Fig. 219), which is not exceptional since within the genus the angle varies from distinctly less than a right angle to about 115°. There is also some variation within certain species.

For the nearctic species of *Neodiprion* (s. str.) about 35 names appear to be available. To the genus belong all or nearly all of the species described in *Lophyrus* (s. str.) by Leach (11, pp. 120-121), Say (24), Harris (10, pp. 375-377), Fitch (8, pp. 58-60, 63-64), Norton (15, p. 225; 16), Cresson (5, p. 25), Dyar (7), and Rohwer (17). Other species have been described in the genus *Neodiprion* by Rohwer (18, 19, 20), Middleton (13, 14), and Schedl (24). Some of the larval forms have been dealt with by Yuasa (29).

Fortunately, almost all of the nearctic species were described from females. Only *N. edwardsi* (Nort.) and *N. hypomelas* (Roh.) were described from the male sex alone, and, as their types were taken in California and Colorado respectively, these species would seem to merit little consideration in this paper. In the present study, attention has been confined almost entirely to the females, a key being provided for this sex only.

The characters used for the identification of females by the earlier workers were size and colour, together with the number of antennal segments. Later workers described species in considerable detail, noting sculpture, shape of the antennal segments, and form of the scopal pad.

Relative size in the pine-feeding species is not of great importance since most of them are from 6 to 7 mm. long, their size being influenced by rearing conditions. However, *N. lecontei* is normally longer and more robust.

General colour is a character that should be used with caution, since yellow, ferruginous, red, piceous, and black tend to blend from one to another. However, *N. maurus* Roh. and *lanielensis* n. sp. form a distinct colour group, being almost entirely black. *N. lecontei* too is recognizable by the almost uniformly rufo-ferruginous colour of the dorsal areas in the prothorax and mesothorax. Colour pattern is of distinct value and is particularly useful in separating the two black species just mentioned, which differ in the pattern of the hind tibia.

Sculpture and punctation have to be used with great care, particularly so with respect to the head. Some variation occurs in the punctation of the

prescutum, scutum, and scutellum; however, the character is useful, particularly in *nanulus* Schedl, in which the scutellum is almost unpunctate, as in such species as *abietis* Harr. and *scutellatus* Roh.

Considerable variation occurs in the conformation of various areas in the head, notably the foveae, the frontal area, and the postocellar area.

As noted by Middleton (12, 13), the number of antennal segments in both sexes is subject to considerable variation. The form of these segments is of some value in separating species (18, 19, 20) but the characters are difficult to use.

The shape of the scutellum has already been discussed above. The tip of the anterior margin is often produced forward, although this character is not specifically stable. Rohwer (18, 19, 20) used the form of the posterior margin as a character, but the character appears to have little value in the species here reviewed.

The shape of the ninth sternum has been used by Rohwer (18) to differentiate certain species, but the character is of no value in our species.

As already noted, the shape of the scopal pads upon the apex of the ovipositor sheath has been considered by both Rohwer (18, 19, 20) and Middleton (13, 14) of great value in grouping species. By this character our pine-feeding sawflies fall into three distinct groups:

1. Species having the scopa much broader than the space between it and the inner margin of the sheath (*ferrugineus* Midd., *lanielensis* n. sp., *lecontei* Fitch, *nanulus* Schedl, *nigroscutum* Midd., *pinetum* Nort., and *rugifrons* Midd., with its variety *dubiosus* Schedl).
2. Species having the scopa about as wide as the space between it and the inner margin of the sheath, the pad more or less flat on top (*banksianae* Roh., *flemingi* n. sp., and *maurus* Roh.).
3. Species having the scopa about as wide as the space between it and the inner margin of the sheath, the pad sloping steeply away laterally, without a flattened apical area (*swainei* Midd.).

Although the form of the scopal pad adequately serves as a means of grouping the species, yet some variation occurs in its proportions. This is particularly marked in *banksianae*, in which reared series have been studied.

The form of the ovipositor is valuable in identifying species. The shape of the lancet may vary specifically, that of *nanulus* being unusually slender and that of *flemingi* having the posterolateral margin straight rather than convex (Fig. 11). Although some intraspecific variation occurs in the shape of the lancet teeth, in their number within the rows, and in the number of rows of teeth, yet these structures are still of major importance in the recognition of species. Particularly useful is the ventral tooth of the second and of the third row of teeth. In the genus *Empria*, Ross (21) terms these ventral teeth *ventral lobes*. These lobes are enlarged, specialized teeth in *Neodiprion* too, the first occurring upon the first row of teeth that is ventrally complete (Fig. 11). The ventral lobes are sufficiently distinct in *maurus* and *nanulus* to permit these species being identified by these parts alone; usually it is necessary to supplement this character with others, particularly those of the scopal pad. In general, only a little variation occurs in the ventral lobes, except in *rugifrons* (s. lat.).

This evaluation of characters is based upon several thousands of specimens, nearly all reared from larvae. The bulk of these belong to *rugifrons*, *nanulus*, *banksianae*, *lecontei*, and *swainei*. Sixty-six female specimens of *maurus* were examined, 18 of *lanielensis* and 25 of *pinetum*, five of *nigroscutum*, and one each of *ferrugineus* and *flemingi*.

No attempt has been made to present the complete synonymy of the various species. The purpose has been to identify the *Neodiprion* species feeding on pines (particularly red and jack pine) in Eastern Canada.

Key to Females

1. Scopal pad much broader than the distance between it and the inner margin of the sheath (Fig. 12) 2
   Scopal pad about as wide as, or narrower than, the distance between it and the inner margin of the sheath (Figs. 13–15) 9
2. Scutellum smooth (very shallowly punctate at lateral and posterior margins); first ventral lobe of lancet large and subquadrate, the second a long and acute ventral tooth (Fig. 3) ..... *nanulus* Schedl (p. 114)
   Scutellum coarsely punctate; first ventral lobe of lancet not unusually large but either dentate or triangular, the second not a long ventral tooth 3
3. Head, prescutum, scutum, and scutellum uniformly rufo-ferruginous, immaculate, the prescutum and scutellum sometimes yellowish; species large and stout, usually much longer than 6 mm.; first ventral lobe of lancet more than twice as long as wide, with a coarse anterior tooth (Fig. 1) ..... *lecontei* (Fitch) (p. 115)
   Head, prescutum, scutum, and scutellum not uniformly rufo-ferruginous, one or more with black; species not unusually large or stout; if first ventral lobe distinctly longer than wide (*pinetum*), then its anterior tooth slender (Fig. 6) 4
4. Body black (sometimes more or less piceous) with whitish markings; hind tibia entirely white; first ventral lobe of lancet not unusually long, the ventral margin sloping ventro-posteriorly (Fig. 10) ..... *lanielensis* n. sp. (p. 115)
   Body extensively ferruginous, often with a large portion black 5
5. First ventral lobe of lancet somewhat longitudinally rectangular, nearly twice as long as high, the anterior tooth long and slender (Fig. 6); lancet with eight or nine rows of distinct teeth ..... *pinetum* (Nort.) (p. 118)
   First ventral lobe weakly developed (Fig. 4), subtriangular (Fig. 9) or about as long as high, the anterior tooth coarse and short (Fig. 7) 6
6. Lancet ventrally emarginate at and behind the second row of teeth, the first ventral lobe weakly developed (Fig. 4); first lancet row with about four teeth, the second row with coarse and quite uneven teeth ..... *nigroscutum* Midd. (p. 118)
   Lancet ventrally not emarginate at the second row of teeth, the first ventral lobe well developed; teeth of second lancet row at least fairly even 7
7. First ventral lobe of lancet small and equilateral, pointing ventrally (Fig. 9) ..... *ferrugineus* Midd. (p. 119)
   First ventral lobe of lancet large, swollen posteriorly, ventrally straight or emarginate, anterodorsally toothed (Fig. 7) 8
8. Abdomen black or blackish ..... *rugifrons* Midd. (s. str.) (p. 119)
   Abdomen rufous or ferruginous ..... *rugifrons* var. *dubiosus* Schedl (p. 119)
9. Scopal pad sloping steeply, the inner margin much higher than outer and meeting it at an angle of about 40° (Fig. 14); second ventral lobe bidentate, sloping anteroventrally (Fig. 8); prescutum fairly closely and evenly punctate ..... *swainei* Midd. (p. 120)
   Scopal pad with inner margin not much higher than outer, sometimes meeting at an angle of about 80° (Figs. 13, 15); second lancet lobe not bidentate, without an antero-ventral slope, except in *flemingi* (Fig. 11) which has the prescutum sparsely and unevenly punctate 10
10. First ventral lobe of lancet subquadrate, swollen, nearly as high as long, with postero-ventral margin distinctly angulate (Fig. 2); body black with whitish markings, the vertex sometimes piceous ..... *maurus* (Roh.) (p. 121)
    First ventral lobe of lancet either the size of the second lobe or else much longer than high; body extensively reddish 11

11. First ventral lobe of lancet rounded, about as high as long, not larger than the second (Fig. 11); scutellum coarsely and evenly punctate..... *flemingi* n. sp. (p. 121)
First lobe of lancet rectangular, twice as long as high, much longer than the second (Fig. 5); scutellum with anterior portion sparsely, finely punctate..... *banksianae* Roh. (p. 122)

Neodiprion nanulus Schedl

(Fig. 3)

Neodiprion sp., the black-headed jack pine sawfly, Schedl, Ann. Rept. Entomol. Soc. Ontario for 1930, 61 : 75, 1931.

Neodiprion nanulus Schedl, Z. angew. Entomol. 20 : 449–460, 1933; Mitt. deut. entomol. Ges. 6 : 41–44, 1935; Z. angew. Entomol. 24 : 51–70, 181–215, 1937.

Neodiprion sp. undescr., Brown, Ann. Rept. Entomol. Soc. Ontario for 1939, 70 : 98, 1939; ibid., Can. Dept. Agr., Ann. Rept. Forest Insect Survey for 1939 : 13, 19, 1940; ibid. for 1940 : 18, 1941; ibid. for 1941 : 14, 1942.

In 1933 Schedl (26) first used his names *nanulus* and *dubiosus* in a paper upon the application of Dyar's Law to these species and to *swainei*. Although Schedl evidently had no intention of validating these names, yet he did so by stating (*a*) that his two species were taken on *Pinus Banksiana* in northwestern Ontario; (*b*) that both of his species had five larval instars and *swainei*, six, and (*c*) that certain statistical differences in the width of the head capsule occurred within each of the various instars of the three species. From the nomenclatorial viewpoint, this is sufficient to constitute differentiation between species and the names *dubiosus* and *nanulus* therefore are technically available, dating from 1933.

From the zoological viewpoint, this differentiation is entirely inadequate for the recognition of Schedl's species. Recourse must therefore be made either to the original material or to Schedl's supplementary papers.

All or nearly all of the original material was taken to Germany by Schedl. Should these specimens prove to be lost or otherwise unsuitable for lectotypes, some of his larvae, taken at Biscotasing, Ont., and now in the Canadian National Collection may be eligible. The adult "types", later described as such by Schedl (27), may be regarded as plesiotypes.

In the absence of type material, one should examine Schedl's other papers. From these it is evident that Schedl consistently considered that at Biscotasing there were known to him only three species of *Neodiprion* on jack pine. Two of these had pale-headed larvae (*swainei* and *dubiosus*) and early summer adults. The other had black-headed larvae and fall adults. The latter species was at first considered as apparently *banksianae* but later as a new species, *nanulus*. These data are in agreement with Schedl's plesiotype and with our present knowledge. While there is the possibility of his 1933 larvae being either *banksianae* or a species unknown to us, yet in the absence of type material, there are no reasonable grounds for doubting the correctness of Schedl's association of adult and larval forms.

Some systematists may regard Schedl's 1933 descriptions (26) as insufficient to validate the names *nanulus* and *dubiosus*. In this case, these names will still apply to the same species but date from 1935; the specimens designated

by Schedl (27) in 1935 as holotypes will then stand. However, this course of reasoning is to be deprecated as the term differentiation, as used in the International Code, must nomenclatorially be interpreted as broadly as possible, as in the past, lest greater confusion be created.

The species *nanulus* is easily recognized among the jack pine sawflies by its almost entirely smooth scutellum; the lancet too has the first lobe large and subquadrate and the second quite unusual as a sharp ventral tooth. The lancet usually has nine rows of exceptionally even teeth, but occasionally there are 10. Each lobe posterior to the third is in the form of a single tooth pointing anteroventrally and rounded ventrally.

The species is common and is to be found in Canada from the Maritime Provinces at least as far west as eastern Manitoba and from as far north as English River, Lake Abitibi, and Lake Saint John to as far south as Sarnia and the Eastern Townships of Quebec.

Neodiprion lecontei (Fitch)

(Fig. 1)

Lophyrus Lecontei Fitch, Rept. Noxious Insects N.Y. 4 : 58–59, 1859.

Lophyrus lecontei Fitch, Dalla Torre, Cat. Hymenoptera, 1 : 295, 1894.

Neodiprion (Neodiprion) lecontei (Fitch), Rohwer, Proc. Entomol. Soc. Wash. 20 : 84, 1918 (genotype).

Neodiprion lecontei (Fitch), Middleton, J. Agr. Research, 20 : 741–760, 1921; Yuasa, Illinois Biol. Monogr. 7(4) : 47, 1922; Twinn, Ann. Rept. Entomol. Soc. Ontario for 1934, 65 : 124, 1935; ibid. for 1935, 66 : 91, 1936; ibid. for 1936, 67 : 84, 1937; Ross Illinois Biol. Monogr. 15(2) : 58, 151, 1937; Atwood, Can. Dept. Agr., Div. Forest Insects, Spec. Circ. Jack pine sawflies, 1938; Brown, Ann. Rept. Entomol. Soc. Ontario for 1937, 68 : 14, 17, 1938; Twinn, ibid. : 83, 1938; Reeks, ibid. for 1938, 69 : 26, 1938; Twinn, ibid. : 131, 1938; Brown, ibid. : 46, 50, 1938; Brown, ibid. for 1939, 70 : 97, 1939; Twinn, ibid. : 123, 1939; Benson, Bull. Entomol. Research, 30 : 340–342, 1939; Brown, Can. Dept. Agr., Ann. Rept. Forest Insect Survey for 1939 : 19, 1940; ibid. for 1940 : 19, 1941; ibid. for 1941 : 14, 1942.

The holotype of *lecontei* is in the United States National Museum and has not been seen by the writer. However, Middleton (12) has characterized the larval instars and the adults of this very distinctive species. The uniformly rufo-ferruginous colour of the head and of the dorsum of the thorax is diagnostic, as well as the stout and usually longer body. The lancet has 9 or 10 rows of even teeth, those of the first two rows, finer. The first row of teeth is long, almost reaching to the ventral margin of the lancet. The first three ventral lobes are angulate posteriorly (Fig. 1), the lobes beyond lacking the posterior angle.

The species is distributed throughout the length of the Appalachians, occurring as far south as Florida and Mississippi (12). In Canada, the species is to be found from the Atlantic coast to Lake Superior and as far north as Kipawa Lake, Que. in the Ottawa valley and Lake St. John, Que.

Neodiprion lanielensis n. sp.

(Figs. 10, 12)

Female. Length 7 mm. Head coarsely punctate; labrum sparsely, indistinctly punctate; clypeus with basal two-thirds irregularly, sparsely, and coarsely

punctate; apical third of clypeus smooth, somewhat depressed and broadly arcuate; median fovea small, round, fairly deep; vertex coarsely and sparsely punctate; vertical furrows distinct but not deep; postocellar area weakly arched, twice as wide as long, medially undepressed; antennae with 18 segments, third and fourth ones subequal in length and distinctly longer than wide at the base; antennal rami shorter than basal antennal width, the middle rami weakly emarginate in posteromedian view.

Thorax with coarse, irregular, and partially confluent punctation on mesoscutum and scutellum; mesoscutum laterally with much finer and closer punctation; angle of anterior margin of scutellum slightly obtuse, the apex not differentiated; scutellum posteriorly angular; cenchri separated by two-thirds of their own length; metascutellum closely, coarsely punctate, with precipitous anterior margin; mesoepisternum closely and coarsely punctate; hind basitarsus scarcely two-thirds as wide as long.

Abdomen shining, sparsely and indistinctly punctate; seventh sternum with emargination one-third as deep as wide, moderate in size; nates microscopically reticulate and very sparsely punctate; scopal pads three times as long as wide, flat above, inner margin subcarinate and separated from inner sheath margin by one-third the width of the pad (Fig. 12); lancet with 10 rows of uniformly large teeth; first row of lancet teeth long, reaching nearly to ventral margin of lancet; first ventral lobe of lancet anteriorly toothed and posteriorly swollen, the following lobes sharply pedate and pointing anteroventrally (Fig. 10).

Colour black; vertex with piceous spot lateral to vertical furrows; apical third of both labrum and clypeus yellow-brown; adjacent margins of second and third antennal segments dull yellow; palpi, apices of coxae and of femora, trochanters in part, tibiae, tarsi, and lateral stripe on abdomen pale yellow; apices of hind tibiae stained with ferruginous.

Male. Length 7 mm. In general agrees with description of female except median fovea larger, deeper, and longer; postocellar area more strongly arched; antenna with 20 segments; punctures of prescutum and scutum finer and closer; hind basitarsus slightly more than twice as long as wide. Colour black; labrum, clypeal apex, second antennal segment apically and tegulae apically, yellow-brown; vertex lateral to vertical furrows, black; legs ferruginous with coxae (except apices) black, the femora and hind tibia reddish; sternites strongly stained with red; hypandrium reddish ferruginous except the black base.

Holotype: ♀, Laniel, Que., 15-II-1941 (C. E. Atwood, 12140-92) reared on *Pinus Banksiana;* No. 5357 in the Canadian National Collection, Ottawa.

Allotype: ♂, same data except 18-II-1941.

Paratypes: Host *Pinus Banksiana:*—3 ♂♂, 1 ♀, Laniel, Que., 13 to 19-II-1940 (C. E. Atwood, 12140-92); 1 ♀, Petawawa Forest Reserve, Ont., 26-II-1940 (C. H. Zavitz, S. 133); 2 ♀♀, Laniel, 10 to 11-VII-1931 (M. B. Dunn). Host *Pinus resinosa:*—7 ♀♀, Laniel, 15 to 18-II-1941 (C. E. Atwood, 12136-

66); 1♂, 1♀, Laniel, 21-VI-38 (C. E. Atwood, 12111-8), both on same pin; 1♂, 1♀, Laniel, 15-II-41 (C. E. Atwood, 12136-76); 3♂♂, Norway Bay, Ont., 4 to 7-II-1941 (Forest Insect Survey 3152); 1♀, Red Pine Chute, P.Q., 16-I-42 (Forest Insect Survey 5104). Host probably *Pinus Banksiana*:—1♀, Mattawa, Ont., 4-II-1941 (Forest Insect Survey 2621). Host not recorded:—1 ♀, Bathurst, N.B., 18-VII (no year given), (J. N. Knull). Specimens emerging in January and February were incubated. Paratypes have been placed in the collections of the United States National Museum (Washington, D.C.) and of the Illinois State Natural History Survey (Urbana, Ill.). The remainder are in the Canadian National Collection.

Among the female paratypes certain colour differences were noted, these being the vertex entirely black, labrum piceous, clypeus apically ferruginous, mesoepisternum below tegulae yellow to piceous, hind tarsus and lateral abdominal stripe stained with ferruginous. The Bathurst specimen and one from Laniel (Dunn) have the whole of the vertex, of the pronotum, and of the mesoepisternum piceous. The form of the ventral lobes of the lancet varies a little. The male paratypes vary in colour somewhat similarly to the females, some specimens being much paler than others.

The females of *lanielensis*, being black with white markings, may be readily separated from the majority of *Neodiprion* species. The species with black females are *compar* Leach, *dyari* Roh., *eximinus* Roh., *gillettei* Roh., *lateralis* Cress., *maurus* Roh., *mundus* Roh., and *pratti* Dyar.

The only description of *compar* is brief and is inadequate for recognition, although it agrees with *lanielensis* in the colour of the body, tibiae, and tarsi. The types are abroad. The type locality is Georgia, which is not sufficiently south to eliminate this species from consideration (*lecontei* has been recorded (12) as occurring as far south as Louisiana, Mississippi, and Florida). There is therefore a small possibility of *lanielensis* being a synonym of *compar*.

The names of the remaining species with black females can be eliminated with a very fair degree of confidence by using such characters in the descriptions as seem most stable. *N. dyari* is easily separated by its almost impunctate posterolateral margin of the prescutum (13 : 171), *eximinus* by its ferruginous head (19), and densely punctate scutellum (14 : 79), *gillettei* by its flagellar rami being twice as long as the segmental width with the basal ramus the longest, *lateralis* by its femora being black (except at the apex), *maurus* as shown in the key above, *mundus* by the almost or entirely impunctate scutellum, and *pratti* by the larva having a pale head and feeding on cedar.

The writer has much pleasure in naming this species *lanielensis* in honour of those forest entomologists who studied the genus *Neodiprion* biologically at the Laniel laboratory in Quebec.

Neodiprion pinetum (Nort.)

(Fig. 6)

Lophyrus le Contii [*sic*] Kirkpatrick (*nec* Fitch), Ohio Farmer, 9 : 269, 1860 (*fide* Rohwer).
Lophyrus pinetum Norton, Trans. Am. Entomol. Soc. 2 : 328, 1869.
Lophyrus abboti Riley (*nec* Leach), Ann. Rept. Insects Missouri, 9 : 29, 1877 (*fide* Rohwer).
Lophyrus pinetorum Dalla Torre, Cat. Hymenoptera, 1 : 297, 1894 (emend.).
Neodiprion pinetum (Nort.) Rohwer, Proc. Entomol. Soc. Wash. 20 : 87, 1918, (neotype); Yuasa, Illinois Biol. Monogr. 7(4) : 47, 1922; Middleton, Can. Entomol. 65 : 82, 1933; Brown, Ann. Rept. Entomol. Soc. Ontario for 1937, 68 : 14, 1938; ibid. for 1938, 69 : 46, 1938; ibid. for 1939, 70 : 97, 1939; ibid., Can. Dept. Agr., Ann. Rept. Forest Insect Survey for 1939 : 15, 1940; ibid. for 1940 : 24, 1941.

The type of *pinetum* has been either lost or ruined according to Rohwer (18), who designated for this species a neotype reared on white pine in Pennsylvania. The species is currently believed to be distinctive in its last feeding stage. The adults used in describing this species have been reared from such larvae.

The original description has been supplemented by Rohwer (18) and Middleton (14 : 82). The scopal pad is about three times as long as wide and is considerably broader than the space between it and the inner margin of the sheath. The lancet has been described in the key.

This species is not particularly common. In Canada it occurs north as far as Sault Ste. Marie, Lake Baskatong, Quebec City, and Gaspé, and south as far as Lake Ontario and the Bay of Fundy; it also has been taken in Newfoundland. Its Canadian distribution is rather similar to that of *lecontei*.

Neodiprion nigroscutum Midd.

(Fig. 4)

Neodiprion (Neodiprion) nigroscutum Middleton; Can. Entomol. 65 : 80–81, 1933.

The type material available for study consists of a single female paratype. The holotype was reared on jack pine at Biscotasing, Ont., by Schedl, the cocoon was spun on September 12 and the adult emerged on December 17. The holotype is labelled "Black head, type ♀", the colour presumably referring to the head of the larva. The Ottawa paratype emerged on December 18. From the two dates of emergence, it is to be assumed that the adults were incubated and would normally emerge in early summer; this is borne out by subsequent rearing data.

The species appears to be quite rare; indeed the writer has only seen four other specimens, all reared by Dr. J. W. M. Cameron at Chalk River, Ont. These emerged in an incubator in February.

The adults are distinct from the other pine-feeding species in the form of the lancet, its ventral edge emarginate at the second row of teeth, which is therefore without a ventral lobe. The lancet basally has the dorsal and ventral margins strongly divergent but this may not be a stable character.

Neodiprion ferrugineus Midd.

(Fig. 9)

Neodiprion (Neodiprion) ferrugineum Middleton; Can. Entomol. 65 : 82–83, 1933.

A single paratype of this species is available for study. The holotype labels bear the date "10.VII.30", and "Type, brown ♀", the colour presumably referring to that of the larval head. The paratype at Ottawa has labels bearing the date "8.6.1930" and the word "row"; the latter refers to the method of oviposition. The type series was reared on jack pine at Biscotasing, Ont.

Although the data for the type series agree with the life history of *rugifrons* and although our single paratype does resemble that species, yet the difference in the shape of the first lancet lobe suggests that the species may be distinct. This lobe is small and triangular, without the coarsely rounded anterior tooth found in *rugifrons*. Considerable variation in the form of this lobe occurs in *rugifrons* but a lobe similar to that of *ferrugineus* has not been found in examining many reared series of *rugifrons*. The original descriptions of these two species do not differ sufficiently to permit confident separation of them. The names should, however, be kept separate for the present.

Neodiprion rugifrons Midd. (s. lat.)

(Fig. 7)

Neodiprion sp., the brown-headed jack pine sawfly, Schedl, Ann. Rept. Entomol. Soc. Ontario for 1930, 61 : 75, 1931.

Neodiprion (Neodiprion) rugifrons Middleton; Can. Entomol. 65 : 79–80, 1933.

Neodiprion dubiosus Schedl; Z. angew. Entomol. 20 : 449–459, 1933; ibid, Mitt. deut. entomol. Ges. 6 : 39–44, 1935; ibid., Z. angew. Entomol. 24 : 51–70, 181–215, 1937; Brown, Ann. Rept. Entomol. Soc. Ontario for 1938, 69 : 46, 1938; ibid. for 1939, 70 : 98, 1939; ibid., Can. Dept. Agr., Ann. Rept. Forest Insect Survey for 1939 : 13, 1940; ibid. for 1940 : 12, 1941; ibid. for 1941 : 8, 1942. **New synonymy.**

With considerable doubt, the writer has placed *dubiosus* as a colour variety of *rugifrons*, rather than as a distinct species. Up to the present the difference in colour has not been correlated with any stable morphological characters, although long series of reared material show a slight tendency for the two forms to merge. It is of interest to note that Schedl's plesiotypes included two specimens having the abdomen mainly black, although this was not mentioned by Schedl (27) in his notes on the variations in *dubiosus*.

The validity of the name *dubiosus* and its identity have already been discussed under *nanulus*, it being concluded that the name dates from the larval description of 1933 and that Schedl's series of adult "types" should be regarded as plesiotypes. The holotype of *dubiosus* not being designated, the interpretation of Schedl as the first reviser (28) has been accepted.

The holotype of *rugifrons* has not been seen by the writer but a paratype has been studied. The type series of *rugifrons* and the plesiotypes of *dubiosus* bear labels with unpublished data of importance. The holotype of *rugifrons* and the paratype at hand have Ottawa incubator labels recording the adult

emergence on Dec. 19, 1930, and a yellow label with "Row" or "Row type". The "holotype" of *dubiosus* and one other female of the series emerged on June 13, 1931, the other females on June 8, 17 (2), 26, 29, and July 1, while the two males emerged on June 8 ("allotype") and July 1. These data agree with the life history of *dubiosus* as given by Schedl (25, 28), in which the adults are described as emerging in early summer and ovipositing by the row method, their larvae being pale headed. The larvae of both series were taken by Schedl on jack pine at Biscotasing, Ont.

As suggested above, this species (or group) varies considerably in some morphological characters; these include the length of the antennal rami, the slope of the metascutellum, the proportions of the teeth of the tarsal claws, the dorsal profile of the base of the lance and the proportions of the first and second ventral lobes in the lancet. These characters, however, do not appear to be correlated with colour.

In Canada *rugifrons* (s. lat.) extends throughout the jack pine areas of Ontario and Quebec, as far east as New Brunswick and west to Prince Albert, Sask.

Neodiprion swainei Midd.

(Figs. 8, 14)

Neodiprion sp., the jack pine sawfly, Dunn, Can. Dept. Agr., Div. Forest Insects, Spec. Circ. The jack pine sawfly, 1931.

Neodiprion sp., the twin-egg sawfly, Schedl, Ann. Rept. Entomol. Soc. Ontario for 1930, 61 : 75–76, 1931.

Neodiprion (Neodiprion) swainei Middleton; Proc. Entomol. Soc. Wash. 33 : 171–174, 1931.

Neodiprion swainei Middleton; Can. Entomol. 65 : 79, 1933; Schedl, Z. angew. Entomol. 20 : 449–459, 1933; ibid., Mitt. deut. entomol. Ges. 6 : 44, 1935; Twinn, Ann. Rept. Entomol. Soc. Ontario for 1934, 65 : 123, 1935; ibid. for 1936, 67 : 84, 1937; Schedl, Z. angew. Entomol. 24 : 51–70, 181–215, 1937; Atwood, Can. Dept. Agr., Div. Forest Insects, Spec. Circ. Jack pine sawflies, 1938; Brown, Ann. Rept. Entomol. Soc. Ontario for 1937, 68 : 14, 1938; ibid. for 1939, 70 : 98, 1939; ibid., Can. Dept. Agr., Ann. Rept. Forest Insect Survey for 1939 : 13, 1940; ibid. for 1940; 12, 1941; ibid. for 1941 : 8, 1942.

Although the holotype of this species has not been seen by the writer, yet available for this study were two male and three female topoparatypes. The type series was reared in the Mont Laurier district of Quebec Province, the host being jack pine and the collector M. B. Dunn. Although the original description of *N. swainei* did not include the date of emergence, Dunn (6) reported this to be the latter part of June.

The females of this species are reddish and have a scopal pad that is unique among the species of pine feeders studied in this paper. These pads are three times as long as wide and slope steeply from each other; their adjacent sides are high and vertical as well as closer to the inner margin of the sheath than to the outer margins of the pad. The pads, too, are unusually well defined for they are distinctly raised above the level of the remaining, ridged portion of the scopa. The same general form of pad occurs in Maine specimens of *pinus-rigida* Nort. identified by Middleton. However, *pinus-rigida* is distinct in having its pad narrower, not more than a third as wide as the

distance between it and the inner margin of the sheath. In *swainei* the lancet has nine rows of teeth, the latter being unusually wide apart in the rows. The form of the ventral lobes (Fig. 8) is valuable in checking determinations.

In Canada this species has been taken as far north as Rimouski, Lake St. John, and the Kapuskasing River, east to the Maritime Provinces, west as far as southeastern Manitoba, and south to Sault Ste. Marie and Ottawa.

Neodiprion maurus Roh.

(Fig. 2)

Neodiprion maura Rohwer, Proc. Entomol. Soc. Wash. 20 : 89–90, 1918.

Although the type material of *maurus* has not been examined by the writer, this species seems quite distinctive. The type series of *maurus* was reared on jack pine in Wisconsin, close to Lake Superior; our Canadian material has the same host and also is found in the Lake Superior region.

This species is distinctive among our pine-feeders by the female being black with the hind tibia white but somewhat ferruginous apically. The other species of *Neodiprion* having black females have been discussed under *lanielensis* and can be separated from *maurus* by the same characters.

Our adults agree with the original description of *maurus* and show the same range of variation in colour. Other differences occurring among our series of females include the clypeus varying from black to almost entirely yellow, the hind tibia usually ferruginous with a longitudinal black stripe, and the postocellar area usually arched. Among the males the hind tibia is usually pale with a black anterior line. The lancet has sometimes 10 but usually 11 rows of teeth, those of the second and third rows uniform. The bloated form of the first ventral lobe (Fig. 2) is unusual, as are the lobes posterior to the third; each of the latter consists of an anteroventral, sharply acute, ventrally curved tooth.

In Canada the species has been taken in the triangle formed by a line from Kenora to Lake Abitibi and the upper St. Maurice valley, and extending south as far as the north shore of Lake Huron.

Neodiprion flemingi n. sp.

(Figs. 11, 13)

Female. Length 7 mm. Head sparsely, rather finely punctate; labrum shining, almost smooth; clypeus with apex broadly and somewhat angularly emarginate, shining; supraclypeal area coriaceous; median fovea small, circular; frontal area raised, flat, sparsely punctate, with faint and irregular rugulae; vertex sparsely punctate, vertical furrows subobsolete, postocellar area twice as wide as long and weakly arched; antennae with 17 segments; basal width of proximal flagellars slightly shorter than length of segment and slightly longer than length of ramus; ramus of basal flagellar shorter than the others.

Thorax dorsally shining; prescutum sparsely and finely punctate, scutum more closely so; scutellum uniformly covered with very coarse, separated punctations that are irregular in size and shape; scutellum with anterior margin forming scarcely more than a right angle, posteriorly rounded; cenchri separated by two-thirds of their length; metascutellum coarsely punctate, anteriorly with precipitous slope; mesoepisternum shallowly and discontinuously punctate; apical width of hind basitarsus equal to length of longer tibial spur and three-fifths the length of the basitarsus excluding pad.

Abdomen shining, impunctate except sparsely on the microscopically reticulate nates; seventh sternum with emargination three times as wide as deep; scopal pad three times as long as wide, as wide as the space between pad and inner margin of sheath; lancet with nine rows of teeth, the first ventral lobe anterodorsally and posteroventrally swollen.

Colour ferruginous; coxae, trochanters, base of tibiae, venter and lateral stripe of abdomen, whitish; labrum yellow; pronotum and scutellum yellowish-ferruginous; femora rufo-ferruginous; mesosternum posteriorly dark; metapleuron stained with black; antennae, dorsal and lateral margins of frontal area broadly, prescutum, scutum, and abdomen dorsally, black.

Holotype: ♀, Red Pine Chute, Kipawa, Que.; 5-II-1941 (incubator); collected by H. S. Fleming on *Pinus resinosa;* No. 5358 in the Canadian National Collection at Ottawa.

This species is described from a single female taken as a larva by H. S. Fleming and reared with sister larvae under the Forest Insect Survey No. 2705. After emergence from the incubated cocoon, the female oviposited on red pine. The larvae upon which the larval description is based were offspring of this specimen.

Neodiprion flemingi can be separated by this description from all of the adequately described species of the northeastern portion of North America. It can be distinguished from specimens of *N. americanus* Leach and from *N. dyari* Roh. (as determined by Sandhouse and Middleton, respectively) by the first ventral lobe of the lancet in the latter two species being longitudinally subquadrate rather than triangular. *N. flemingi* also appears close to *N. eximinus*, which was reared from Wisconsin red pine, but the latter is distinguished by the densely punctate scutellum (14 : 79), the closely punctate prescutum, and the long spur of the hind tibia.

Neodiprion banksianae Roh.

(Figs. 5, 15)

Neodiprion n. sp., Graham, J. Econ. Entomol. 18 : 337–341, 1925.

Neodiprion banksianae Rohwer, Proc. Entomol. Soc. Wash. 27 : 115–116, 1925; Graham, Principles of forest entomology : 161–165, 1929.

Neodiprion sp., the black-headed jack pine sawfly; Schedl, Ann. Rept. Entomol. Soc. Ontario for 1930, 61 : 75, 1931 (in part).

Neodiprion (Neodiprion) ontariocnsis Middleton, Can. Entomol. 65 : 83–84, 1933. **New synonymy.**

Neodiprion banksiana [*sic*] Rohwer; Middleton, Can. Entomol. 65 : 83, 1933.

Neodiprion nanulus Schedl; Brown, Ann. Rept. Entomol. Soc. Ontario for 1939, 70 : 98, 1939; ibid., Can. Dept. Agr., Ann. Rept. Forest Insect Survey for 1939 : 13, 1940; ibid. for 1940: 12, 1941; ibid. for 1941 : 8, 1942.

The holotypes of *banksianae* and *ontarioensis* have not been examined by the writer but a paratype of each species is available. The proposed synonymy is based mainly upon a study of long series of reared specimens, taken chiefly in Ontario and Quebec. According to Middleton (14), *ontarioensis* differs from *banksianae* in body size and in the number of antennal segments, as well as in the colour of the head, scutellum, and abdomen. However, reared series show that these characters vary sufficiently to account for the disagreements between the descriptions of Rohwer and Middleton for this species. The available paratype of *banksianae* differs from almost all of our Canadian specimens in being distinctly lighter in colour and in having a scopal pad slightly wider than the distance between it and the inner margin of the sheath; however, a few individuals from reared series agree with this paratype of *banksianae*.

Since no date of emergence has been published for the holotype of *ontarioensis*, it is pertinent to note here that according to a label on this specimen, the cocoon was spun on July 10, 1930; adult emergence would then occur in the fall. A second label has the words "black head, ♀", the colour evidently referring to the larva. These data agree with the biology of *banksianae*, as published by Graham (9).

The proposed synonymy is supported by the agreement of larval descriptions and other data of Graham with those of Atwood. Additional evidence is furnished by some Minnesota specimens (St. Paul, Sept. 1929, L. W. Orr), which agree in colour and scopal form with the common Canadian specimens. The type locality of *banksianae*, too, is Itasca Park, Minn.

The females of *banksianae* usually can be recognized by the pale general body colour, the coarsely punctate scutellum and by the usually elongate scopal pad which is about as wide as the distance between it and the inner margin of the sheath. The scopal pad usually is four to five times as long as wide and then is a distinctive character, but those few specimens in which the pad is proportionately broader should be examined to see that the first lancet lobe is longitudinal and that the lancet has 10 or 11 rows of teeth.

The species has been found in Canada to extend from New Brunswick to eastern Manitoba. It is one of our more abundant species.

Identification of Immature Forms

By C. E. Atwood

Larvae

The forest entomologist is more likely to encounter species of *Neodiprion* in the larval stage than as adults. Unless he can identify the larvae when found or at least before they spin cocoons, it is necessary for him to rear the insects to the adult stage, which may involve a delay of nearly a year, as well as a good deal of work, before he has any prospect of securing a name for

the insect. If the larva is parasitized or dies from some other cause, all the work will be lost and no name will have been secured. With these facts in view, the following key has been prepared in order to facilitate the identification of larvae in the field, using a hand lens, which in general is sufficiently powerful to show the characters used in the key.

In using the key, a certain amount of care is necessary in selecting material since not only larval coloration but also the type of egg scar pattern and details of the seasonal life history have been used in the attempt to indicate the identity of each species. Therefore, larvae should be studied in conjunction with certain data that often are a necessary aid to their identification. The nature of these data will appear from the following sentences and from the key. In general, preserved material is hard to determine with certainty and living material should be used when possible. Even then, the key characters based on larvae alone are not always as reliable as could be wished, but fortunately, the larvae that resemble each other most closely in appearance are readily distinguished by the egg scar pattern characteristic of the species and by seasonal histories. The following rules may therefore be suggested in connection with the use of these keys.

1. Use living larvae in the last feeding stage, rearing them on the proper food if necessary, until that stage is reached.

2. When colonies are collected in the field, try to secure the needles bearing the eggs from which the larvae hatched.

3. Examine carefully each individual larva in a colony, as mixed lots are often found on the same tree or even on one branch.

4. If any doubt exists regarding the life history, keep cocoons spun by the larvae under proper conditions in order to find out whether the adults emerge in the fall or spring.

5. Until thoroughly familiar with the larvae of each species, confirm determinations by rearing adults and checking their identity by means of the keys and descriptions in this paper, or by submitting them to a specialist.

Knowledge of the life histories of these species is still very incomplete; many facts are not known at all, while tentative opinions on other aspects rest on insufficient evidence and must be verified or disproved by further work.

Oviposition and Seasonal History

The eggs, egg pockets, and oviposition habits of certain species of *Neodiprion* have been described by Middleton (12), Burke (3), and other authors. In general, the egg pockets are slits made in the edge of the needle by the ovipositor of the female sawfly and viewed from the side are roughly oval or shoe-shaped, the shape being somewhat variable for each species. However, it was found that each species makes a characteristic "egg scar pattern" because of the spacing of the eggs in relation to the length of the needle and that these patterns can sometimes be used as diagnostic characters for the species. The spacing of the eggs in the needle and on the tree also corresponds with the

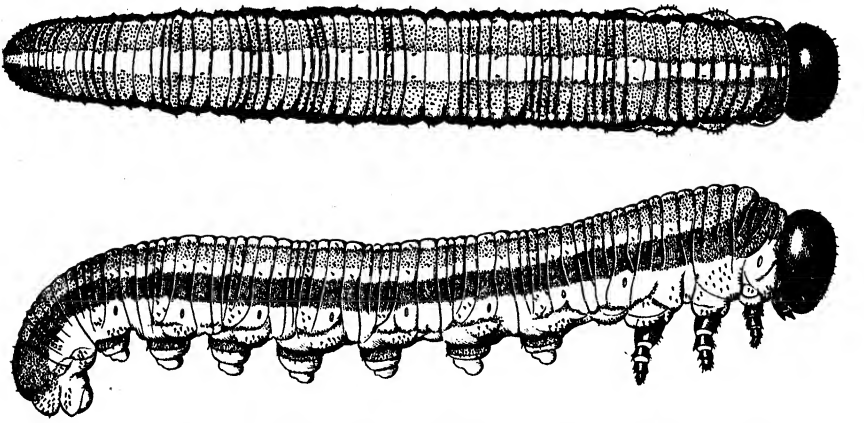
habits of the larvae in regard to gregarious or non-gregarious feeding. In general, each female lays all her eggs in a single clump of needles on one twig or on closely adjacent twigs, but some species do not do so.

KEY FOR THE DETERMINATION OF *Neodiprion* LARVAE

(Based on the last feeding stage and other details of the life history)

1. Subdorsal line consisting of a series of black or dark marks, distinctly separated from each other 2
   Subdorsal line practically unbroken 3
2. Head capsule predominantly black ..... *pinetum* (Nort.) (p. 128)
   Head capsule orange to brown ..... *lecontei* (Fitch) (p. 129)
3. Head capsule predominantly black, with or without lighter markings on frons and clypeus 4
   Head capsule orange to dark brown (sometimes black in *swainei*), with or without dark markings on frons and clypeus; if head black, light posterolateral sutures and black patch on epiproct present 9
4. Epiproct with a distinct black or very dark grayish blotch abruptly separated from subdorsal line 5
   Epiproct without a distinct blotch; if darker than subdorsal line merging inperceptibly with it; head with posterolateral suture noticeably marked with lighter colour 6
5. Black patch on epiproct usually with a distinct light median line for most of its length; dorsal line wider than subdorsal on abdomen; supraspiracular line usually broken, sometimes solid; a gray area between mesothoracic legs extending forward almost to prothoracic legs; larvae on trees in spring and early summer; pass winter in egg stage ..... *banksianae* Roh. (p. 130)
   Black patch on epiproct with median line faint or almost absent; supraspiracular line composed of separate spots; dorsal line narrower than or subequal to subdorsal line on abdomen; area between meso- and prothoracic legs unmarked; larvae on trees in July and later; pass winter in cocoon ..... *maurus* Roh. (p. 131)
6. Head in dorsal aspect marked with a light suture on each side of the posterolateral surface; clypeus and frons with a characteristic light colour pattern ..... *lanielensis* Peck (p. 131)
   Head in dorsal aspect without light markings 7
7. Subdorsal and supraspiracular lines light green to light olive green, hypopleural lines almost absent; head with a pale spot at apex of frons ..... *flemingi* Peck (p. 132)
   Subdorsal, supraspiracular, and hypopleural lines olive green, dark greenish gray or sometimes almost black; head without pale spot at apex of frons 8
8. Lighter parts of body, especially in thoracic region usually dirty light grayish with little suggestion of yellow or green; darker stripes dull dark olive green; eggs laid in fall, scars usually separated in needle by their own length or more; larvae on trees in spring and early summer ..... *nanulus* Schedl (p. 133)
   Almost identical in appearance with the above; lighter parts definitely light olive green or yellowish green, not grayish green; the whole appearance of the larvae, when alive, brighter green than *nanulus*; a grayish blotch on venter between mesothoracic and prothoracic legs; eggs laid in spring or early summer, scars separated by less than their own length; larvae on trees in July and later ..... *Neodiprion* sp.* (p. 134)
9. Dark body markings usually black, very conspicuous, supraspiracular line often nearly solid; black mark on epiproct not sharply divided by a median line; cocoons when thoroughly soaked turn reddish or mahogany brown; eggs laid in rows, many per needle; larger ..... *rugifrons* Midd. (p. 135)
   Dark markings often greenish or grayish, supraspiracular line almost never solid; black mark on epiproct divided by a light median line; cocoons remain buff or varnish yellow, seldom any suggestion of reddish; eggs laid one or two per needle, paired with those laid on other needle of the cluster (Fig. 52) ..... *swainei* Midd. (p. 136)

* *Adults agreeing with female N. ferrugineus Midd., the only sex described, have not been reared. On the other hand, only eggs and larvae of the species in Couplet 8 of the key above, with nanulus, are known, and as the larvae were produced parthenogenetically by a female that was accidentally destroyed before it could be examined, the resulting adults were male only. When more larvae of this species are secured and reared so as to produce females, this species may prove to be ferrugineus.*



TEXT FIG. *Dorsal and lateral views of Neodiprion nanulus Schedl, V stage* ♀.

Description of Larvae

In the following descriptions, the plan followed is that of Middleton (12, pp. 742, 743). For convenience of reference, a small part of his paper is quoted below.

"In the description of sawfly larvae, both structurally and for color, it is necessary that particular areas and regions of a segment or body wall be designated and that the designation adopted be applicable to both the thorax and abdomen of the larva in all its stages. Further, the method, or system, should permit by addition, elimination, change in shape, armature, and spotting of folds, areas, or regions, the comparison with other larvae, and at the same time should avoid possible confusion of meaning. The following is a suggestion for such a terminology and is the one used in the succeeding pages.

"An intermediate (second to eighth, inclusive) abdominal segment of *Neodiprion lecontei* consists of tergum, pleurum, and sternum and begins with the transverse tergal fold immediately preceding that above the spiracle.

"The tergum is composed of six transverse folds which are considered as representing four primary divisions (*A*, *B*, *C*, *D*), with one, the third, twice subdivided ($C^{1, 2, 3}$).

"The pleurum is divided into three folds—the dorsal anterior one here called the preepipleurite, the posterior one called the postepipleurite, and a ventral one called the hypopleurite—and two areas, one containing the spiracle and the other, armed with a few spines, posterior to and adjoining that containing the spiracle. The area containing the spiracle is at the lower extremity of fold *B* immediately above the preepipleurite and is termed the spiracular area, while the second area, that posterior to the above and armed with few spines, is below folds $C^{1, 2, 3}$ and is termed the postspiracular area.

"The sternum consists of two transverse folds before the hypopleurites, one between and one behind them. The hypopleurites bear the uropods. . . . . .

"The interpretation of the segmental composition and terminology outlined above is applied to the thorax (Pl. 91, A,D) in the following way. Each of the three thoracic segments (prothorax, mesothorax, and metathorax) is 4-annulate tergally, and the annulations when viewed with reference to ornamentation, shape, position, and relation with one another homologize in order with the primary divisions (*A*, *B*, *C*, and *D*) of the abdomen, the third, *C*, not being subdivided.

"The pleurum is distinctly divided into four lobes, preepipleurite, postepipleurite, prehypopleurite, and posthypopleurite, in all three segments; and the postspiracular area is present, in approximately its relative abdominal position, in the mesothoracic and metathoracic segments, despite the absence or displacement of the spiracle.

"The sternum consists of three small, rather indistinct folds anterior to the leg's basal attachment to prehypopleurite and posthypopleurite.

"Further, the transverse circumference of the larva is divided into longitudinal areas of about equal width (Pl. 91, F).

Tergum or Dorsum

"The tergum or dorsum in the present paper is intended to designate that portion of the larva which is dorsad of the spiracular and postspiracular areas and which is divided into transverse folds or annulets *A*, *B*, *C*, and *D* in the thorax, and *A*, *B*, $C^{1,2,3}$ and *D* in the abdomen.

I[a].—Middorsal, a single longitudinal midtergal line.

I.—Dorsal, a pair of longitudinal tergal regions, one to either side of the middorsal line.

II.—Subdorsal, a pair of longitudinal regions, one to each side of the dorsal regions.

III.—Laterodorsal, longitudinal regions, laterad of subdorsal regions.

IV.—Supraspiracular, longitudinal regions, laterad of latero-dorsal regions.

Pleurum or Latus

"The pleurum or latus designates that portion of the larva between tergum and sternum.

V.—Spiracular, longitudinal regions, one to each side of the larva and ventrad of the supraspiracular regions, with the abdominal spiracle situated therein in most sawfly larvae, including *Neodiprion lecontei*.

VI.—Epipleural, longitudinal regions ventrad of spiracular.

VII.—Pleural, longitudinal regions ventrad of epipleural.

VIII.—Hypopleural or lateroventral, paired longitudinal regions, in which are situated the hypopleurites, one to either side of the sternum and ventrad of the pleural regions.

Sternum or Venter

"The sternum or venter designates that portion of the larva beneath the body between the uropods. The ventrad projection of the uropods places them with reference to the position they occupy in relation to other structures in the adventral longitudinal areas.

IX.—Adventral, paired longitudinal regions containing the uropods, one protruding from each hypopleurite.

X.—Ventral, a pair of longitudinal sternal regions.

X^a.—Midventral, a single, midsternal, longitudinal line."

The descriptions below are based on the final feeding stage and the colours apply to living material except where otherwise stated. As noted elsewhere, it may not always be possible to identify preserved material by means of these keys and descriptions unless certain details of the life history are also known and every effort should be made to secure these when the larvae are collected.

Neodiprion pinetum (Nort.)

Head. Head capsule black, shining, with brownish areas at articulation of mandibles and on adfrontals; proximal third of clypeus dark brown to black, distal two-thirds straw colour marked on each side by a somewhat irregular blotch of black or brown which projects downward from the dark basal third; labrum dark reddish brown to black, mandibles black at base, reddish brown distally, other sclerotized portions of mouth parts reddish brown to black.

Thorax. Prothorax white or very pale yellowish, immaculate except for sclerotized sternal neck plates, prehypopleurites, and leg joints which are black or very dark brown. Meso- and metathorax with a subdorsal black spot extending over *A* and *B*, and a supraspiracular black spot on *B*, *C*, and the postspiracular area. Sternal regions as in prothorax with the exception, of course, of neck plates.

Abdomen. Same colour as thorax with the following markings: a black or dark brown subdorsal spot on *A*, *B*, C^1, and sometimes C^2 of segments one to nine, often obsolescent on some; a supraspiracular and spiracular black spot on *B*, C^1, C^2, and postspiracular area of segments one to nine, these spots darker than the subdorsal and persistent even when the latter are almost absent; epiproct with a black patch divided by a light median line.

Food Plants. Almost exclusively white pine in this region.

Oviposition and Seasonal History. Eggs are small, only three or four on a needle and the scars separated by more than three times their own length. Apparently all the eggs of each female are laid on a single twig and the larvae are gregarious; they appear on the trees during July and August and the mature larvae pass the winter in the cocoon among the litter under the tree.

Parthenogenesis. Nothing is known of the parthenogenetic behaviour of this species, and it has not yet been reared in the laboratory.

Neodiprion lecontei (Fitch)

(Figs. 19, 22, 25, 46)

Head. Head capsule orange-brown, with black spots surrounding the eyes; distal half of clypeus rather paler than capsule, especially at centre, but extreme distal edge bordered with darker brown; labrum often darker brown than head capsule, especially at margin; sclerotized portions of mouth parts pale yellowish brown to almost black.

Thorax. The thorax during life varies from deep yellow to pale whitish yellow and is marked as follows: prothorax immaculate above, sternal neck plates, prehypopleurites, and sclerotized portions of legs, black; on meso- and metathorax a subdorsal black spot on *A*, *B*, and *C*, diminishing posteriorly; a supraspiracular and spiracular black spot on *B*, *C*, and the post-spiracular area.

Abdomen. Similar in colour to thorax; a subdorsal black spot extending over *A*, *B*, and C^1 on segments one to nine inclusive; a supraspiracular black spot on *B*, C^1, C^2, and the dorsal part of the spiracular area; black patch on epiproct divided by a light median line. This description fits the great majority of the hundreds of larvae examined and differs from Middleton's description (12) in showing fewer black spots. Some specimens seen have a black spot on the epipleurites of all except the ninth abdominal segment and also on the meso- and metathorax. Very few have been seen with black postepipleural spots or with the black supraspiracular spot on the prothorax, noted by Middleton. Specimens are occasionally seen in which practically the whole abdomen except the epiproct is immaculate.

Food Plants. The favourite host is undoubtedly red pine, although jack pine is sometimes attacked. White pine foliage may be eaten by larvae that have migrated because of starvation but apparently eggs are not laid on it. In nature, only young pines in the open are attacked and larvae are seldom found on trees more than 15 ft. in height.

Oviposition and Seasonal History. The egg scars are made rather close together, separated by about one-third to one-half their own length. From 10 to 30 or more eggs may be laid on a single needle. All the eggs are laid as a rule on one twig and the larvae are very gregarious, feeding in dense clusters. Mature larvae pass the winter in the cocoon under duff and litter or in loose sandy soil, the latter apparently being preferred. Eggs are laid during June and larvae may be on the trees until late September.

Parthenogenesis. Large numbers of adults have been reared from virgin females; these have without exception been males. Some parthenogenetically produced families of larvae have been thought to be rather less vigorous than the sexually produced lots, but no proof of this opinion is available.

Neodiprion banksianae Roh.

(Fig. 51)

Head. Head capsule black, shining; central portion of clypeus slightly paler, whitish or greenish; labrum, mandibles and sclerotized portions of mouth parts black or very dark reddish brown.

Thorax. Pale greenish yellow to pale olive with the following markings: a subdorsal black line extending from Annulet *C* of the prothorax back on to the abdomen; a small gray spot slightly ventrad of the prothoracic spiracle; a large supraspiracular and spiracular black spot across the base of mesothoracic Annulets *B*, *C*, *D*, and the areas immediately adjacent ventrally; on the metathorax a similar spot often connected with the anterior spot by a few small dark spots on metathoracic *A*; neck plates and sclerotized leg joints black, and an irregularly shaped gray ventral area extending from between the mesothoracic legs forward almost to the prothoracic legs; this is sometimes obsolescent.

Abdomen. The subdorsal line is continuous with and similar to the thoracic; the supraspiracular spots are also similar; these may be separate or may be so large both on thorax and abdomen that they practically form a continuous stripe, in which case *A* as well as the other annulets bears a black spot. On the posterior thoracic and anterior abdominal region, the dorsal line is usually wider than the subdorsal, but is narrower on the prothorax and near the epiproct. Abdominal segments two to eight bear a subspiracular dark gray spot on the spiracular and postspiracular areas and the dorsal part of the pre-epipleurite, while on segments one to eight, the postepipleurite is largely black and the hypopleurite bears a gray spot. The epiproct bears a large black patch with a light median line usually dividing it for at least two-thirds of its length. Specimens are occasionally seen in which many of the dark lines and spots are obsolescent.

Food Plants. This insect is primarily a jack pine feeder. However, in a mixed stand of jack, Scots, and red pine near Spencerville, Ont., the Scots pine is now being attacked with some severity and several egg clusters have been taken on red pine. Larvae have been reared from these eggs using Scots and red pine respectively as food and normal adults emerged from the cocoons, no undue mortality being noted.

Oviposition and Seasonal History. This species usually lays three, four, or five eggs per needle, each scar being separated from the next by two to three times its own length, although variations occur. The full complement of eggs is usually laid on one twig and the larvae are gregarious. Shrubby trees on lake shores, rocky ridges, or other situations fully exposed to sun appear to be preferred for oviposition but even fully stocked, vigorous stands are not immune from attack. The larvae hatch in spring from eggs laid the previous fall and most of them are full grown by early July. About two months are spent in the cocoon.

Neodiprion maurus Roh.

This species is practically indistinguishable in appearance from *N. banksianae* Roh., the characters given in the key to larvae being the only ones discovered and these are so slight that some difficulty will be experienced in their use. The two species are, however, readily separated on the basis of their life history; any larvae with black heads, distinct spots in the supraspiracular position, and a distinct black patch on the epiproct should be considered as possibly belonging to this species if taken on jack pine after July 1 in the general latitude of Lake Temiskaming. Fortunately the females are very different from those of *banksianae* and may be told at a glance from that species.

Host. This species has been collected in the field only from jack pine. As it has been rare, material for testing on other hosts has not been available.

Egg Scar Pattern. The eggs are laid three to six per needle; the scars may almost touch each other but are usually separated by a distance of one-fourth to three-fourths their own length. The full complement appears to be laid on a single twig and the larvae are gregarious. They feed during July and August and pass the winter in the cocoon.

Parthenogenesis. No experiments have been carried out because of lack of material. Production of eggs in the laboratory by this species has been meagre.

Neodiprion lanielensis Peck

(Figs. 28, 31, 34, 39, 40, 44, 50)

Head. Capsule black or dark brown with the following markings: a narrow, pale brown, greenish or cream-coloured line borders the occipital foramen and is produced a short distance along the sutures that extend dorsally from the lateral extremities of this foramen; two light spots of similar colour, roughly semicircular, also continuous with the light border around the foramen, are present on each side of the epicranial suture (Fig. 40). A light spot is present around the dorsal point of the frons and occupies part of the frons itself as well as adjacent areas of the epicranium. A whitish area with a sinuate dorsal margin occupies the ventral part of the frons, extends across the genae with short extensions dorsally along the frontal sutures, and is continuous with the narrow border of the occipital foramen. The antennae are usually just outside the dorsal edge of this light area. The clypeus is darkened along the dorsal margin with a darkened area projecting downward on each side and continuous with the dark upper margin. The central area and the laterodorsal portions of the clypeus are whitish; the labrum, mandibles, and sclerotized portions of the mouth parts are dark brown to black.

Thorax. The ground colour of the thorax is pale olive green or yellowish. A broad subdorsal stripe of a darker, brighter green, about the colour of a red pine needle, extends backward from Annulet *C* of the prothorax and is continued on the abdomen. A very faint narrow laterodorsal line with irregular

edges and similar in colour to the dorsal, extends often in an interrupted condition from *A* of the mesothorax on to the abdomen; ventrad of this again is a supraspiracular and spiracular line similar in size and colour to the subdorsal. Sclerotized parts of the sternum are dark brown or black, including the neck plates; on the prothorax two dark patches are present on each side dorsad of the neck plates and a grayish or greenish patch extends forward, diminishing anteriorly, from the mesothoracic and metathoracic prolegs. Two small grayish spots are also present between the prothoracic legs.

Abdomen. The subdorsal and supraspiracular–spiracular lines are continuous with and similar in colour to those of the thorax, and extend on to the epiproct without a break. Posterior to the metathoracic legs a grayish or greenish stripe runs with slight interruptions along the hypopleural areas between the uropods and across the postepipleurites and hypopleurites of each segment to the eighth, where it stops on the eighth hypopleurite. Coloration of the remaining portions of the abdomen is very similar to that of the lighter parts of the thorax. In preserved specimens, the green stripes fade to gray or brownish and the lighter parts to white.

This larva may be distinguished from any others known from this region by the bright pine needle green of the darker stripes and by the conspicuous light markings on the head, especially on the posterior part of the epicranium.

Food Plants. This species seems equally at home on red and jack pine.

Oviposition and Seasonal History. The egg scars are usually separated by less than their own length but seldom touch each other. On jack pine, four or five per needle is most common; on red pine, there is a tendency to lay them in two groups of four or five even when 10 or so are present on a single needle. The eggs are scattered widely over the tree or possibly over several trees, and the larvae are solitary or nearly so. They feed during July and August and pass the winter in the cocoon.

Parthenogenesis. Only males are known from parthenogenetic eggs.

Neodiprion flemingi Peck

(Figs. 18, 21, 24, 43, 47)

Head. Head capsule black, except for the following markings: a light yellowish or greenish spot at the junction of the frontal and epicranial suture; a pale greenish spot occupies the central part of the clypeus (Fig. 43).

Thorax. General colour dull pale green to greenish white, lighter anteriorly; broad subdorsal and supraspiracular stripes extend backward from Annulet *B* of the metathorax, faintly separated by a paler laterodorsal stripe. Sclerotized parts of legs, neck plates, and prehypopleurites black or dark brown.

Abdomen. Dorsal, subdorsal, laterodorsal, and supraspiracular stripes are continuous and concolorous with those of the thorax, becoming paler posteriorly; a slight grayish mark on postepipleurites of segments two to five, venter whitish or cream with a greenish tinge. Preserved specimens fade to a dull ochre.

This larva may be distinguished from any others known in the area under consideration by the paleness of coloration and lack of conspicuous body markings and by the characteristic markings on the front of the head (Fig. 43). It is most likely to be confused with the black-headed form of *N. swainei* Midd. (Fig. 38) or with *N. lanielensis* (Fig. 40) but the facial markings including the black labrum will serve to separate them; if observed in the field, the typical egg scars of *swainei* are an additional means of identification.

Food Plants. This species has been taken only on red pine, but larvae reared in incubators at Ottawa readily ate jack pine foliage.

Oviposition and Seasonal History. The only female whose oviposition has been observed laid 14 eggs on one needle, these eggs being rather close together. Eggs are probably laid in late June or early July; larvae are on the trees until late August or September and the adults pass the winter as larvae in the cocoon. Larvae are gregarious.

Parthenogenesis. Male sawflies have been secured from eggs laid by a virgin female.

Neodiprion nanulus Schedl

(Figs. 26, 29, 32, 45, 53, Text Fig.)

Head. Head capsule shining black; lower median portion of clypeus and a spot at each side lighter in colour; clypeus dark brown to black; mandibles and sclerotized portions of mouth parts dark reddish brown to black; adfrontals and adjacent parts of genae sometimes brown.

Thorax. Prothorax dirty white to pale grayish green (usually with very little suggestion of yellow in living specimens) with markings as follows: on Annulets *B*, *C*, and *D*, a continuous dark grayish green or dark olive green blotch extending from the dorsal line almost to the spiracle; small grayish spots sometimes present in spiracular and subspiracular positions; legs, neck plates, and prehypopleurite black or dark brown; a grayish area sometimes present between prothoracic legs. Meso- and metathorax with a dark stripe in subdorsal position continuous anteriorly with blotch on prothorax; a similar dark stripe in supraspiracular and spiracular area, both these stripes wider than the pale laterodorsal stripe; scattered small gray patches often present in spiracular and subspiracular areas; a grayish band extending from prothoracic to mesothoracic and from mesothoracic to metathoracic legs on each side; the central portion of the venter between the prothoracic and mesothoracic legs nearly always with a grayish mark.

Abdomen. The dark dorsal and supraspiracular stripes are continuous with those of the thorax extending without noticeable interruption on to the epiproct. A pale spiracular stripe, which may be wide or narrow, runs across the central part of the spiracular and postspiracular areas; ventral to this is a dark band which may occupy only the pleural area or may extend from the lower part of the spiracular area to the hypopleurites, with various spots and areas including usually the pre-epipleurites, still pale; this stripe usually stops on the eighth segment and is represented on the ninth only by a small gray

spot; a larger gray spot is usually present on the subanal fold and a similar one on the base of each hypopleurite.

Food Plants. In the Biscotasing-Chapleau area, this species appears to prefer jack pine for oviposition; at Laniel and Mattawa, red pine is preferred although colonies are frequently taken on jack pine. There appears to be no morphological difference between adults of the two populations and although the jack pine larvae are somewhat lighter in colour, this is possibly due directly to the effect of food. Both young and mature pines of both species are attacked, some of the heaviest feeding on red pine having been observed on trees 65 ft. or so in height.

Oviposition and Seasonal History. The egg scar pattern of this species is somewhat irregular. In general, eggs on red pine are separated by about their own length; on jack pine the interval may be somewhat greater or smaller. On both species there is a decided tendency toward irregular grouping, small groups of eggs and single eggs being common. Ten to 15 eggs per needle are commonly found on red pine; five to eight is a common number on jack pine. The females appear to often divide their eggs among several twigs and small groups of eggs, sometimes only one needle on the twig being attacked, are common. The larvae are gregarious during the early stages but tend to disperse when they reach the fourth. Eggs are laid in the fall, spend all winter on the tree and hatch in late May or June. Cocoons are spun very near the surface of the duff.

Parthenogenesis. All adults secured from parthenogenetically produced eggs have been males.

Neodiprion sp.

This larva is almost indistinguishable from that of *N. nanulus* Schedl. In life it is somewhat brighter green in colour; all the specimens examined lack the ventral gray spot between the prothoracic and metathoracic legs; the subanal spot is extremely faint or absent and the dark line in the pleural region is narrow and practically confined to the postepipleurites and the areas immediately anterior and posterior to them; this line has rather sharply defined borders and does not tend to stretch upward on the the pre-epipleurites and spiracular area. Preserved specimens thought to belong to this species should not be determined without supporting data.

Food Plants. This species is known only from jack pine.

Oviposition and Seasonal History. The eggs are laid very close together, most of them practically touching, five to eight per needle being a common number. The larvae are gregarious. Although field observation is lacking, all the available evidence shows that the larvae feed during July and August and pass the winter in the cocoon.

Parthenogenesis. The only family that has been reared was from a virgin female and from this lot four males have been secured.

Neodiprion rugifrons Midd.

(Figs. 16, 20, 23, 41, 42, 48)

Head. Head capsule bright orange to orange-brown, with black eyespots; antennae, and antennal sockets mostly brown; clypeus paler in centre, lower edge darkened; labrum usually concolorous with head capsule, with dark brown border; base of mandibles orange to brownish; tips of mandibles and sclerotized portions of mouth parts dark brown. Most specimens have darkened areas along the upper portion of the frontal sutures but not at the junction of the frontals and epicranial. The adfrontals are also darkened in many specimens.

Thorax. Prothorax yellowish green to light olive with dark markings as follows: a subdorsal black patch on Annulets *B*, *C*, and *D*; a supraspiracular patch on *B* and *D*, and a much larger supraspiracular and spiracular patch on *C*, frequently extending upward so that it becomes continuous with the subdorsal mark; a small subspiracular spot on the spiracular area, often extending on to the pre- and postepipleurite; leg joints and neck plates dark brown and a brownish or grayish patch just dorsal to the neck plates on each side. The subdorsal marks appear brownish in preserved specimens while the more ventral ones are black or grayish. A dark subdorsal stripe, continuous in front with the marking on the prothorax, extends the full length of meso- and metathorax. A supraspiracular spot may be present on *B*, *C*, *D*, and the areas immediately ventral or may extend completely across all annulets, forming a continuous stripe along the supraspiracular region. The light laterodorsal line between these two darker stripes is usually well-defined except on the prothoracic Annulets *B* and *C*, where it is sometimes partly interrupted by the dark markings.

A black spot is also present immediately in front of each meso- and metathoracic leg; it may extend anteriorly almost to the next leg.

Abdomen. The subdorsal dark stripe noted on the thorax continues uninterrupted across segments one to nine. The supraspiracular markings may be present as patches on Annulets *B*, C^1, C^2, and C^3, with light areas on *A* and *D*, or may be practically continuous, with *B* to C^2 a deeper black than the other annulets. In either case, the patches may extend ventrally to the spiracular and postspiracular areas. A subspiracular gray patch is also present on the ventral portion of the spiracular and postspiracular area of each segment, the dorsal portion of the pre-epipleurite and adjacent parts of this region. The postepipleurite and the lateral portion of the transverse fold in front of the abdominal prolegs are black on segments two to eight; segment one has a less extensive black patch in the corresponding position while on segment nine these markings are absent. In dark specimens the patches may be so enlarged longitudinally as to form an almost continuous epipleural stripe. The epiproct carries a dense black mark with only a trace of a median line.

Food Plants. This species is known only from jack pine.

Oviposition and Seasonal History. The eggs are laid very close together, many almost touching, scars seldom more than one-half their length apart. Ten to 12 eggs per needle are not uncommon. The larvae are very strongly gregarious and when numerous feed in large clusters. They are on the trees during July and August and apparently grow much faster than *N. swainei.* Winter is spent in the cocoon.

Parthenogenesis. Large numbers of males have been reared from the eggs of virgin females; no females have been secured.

Neodiprion swainei Midd.

(Figs. 17, 35–38, 52)

Head. The head capsule colour and pattern in this species are variable, ranging from light orange to black. Four of these variations are shown in Figs. 35 to 38. The typical form has an orange or orange-brown head capsule, black eyespot, a brownish patch along each arm of the frontal suture near its junction with the epicranial; clypeus somewhat darkened at the upper and lower margin with a light central area and a light spot at each side, labrum orange-brown with a dark margin; mandibles orange at base, reddish brown at apex; sclerotized portions of mouth parts black or dark brown. In the forms with darker heads, the vertex and the labrum are usually darkest while the frons, clypeus, and genae retain light markings even in the darkest individuals. The short suture running from the posterolateral margin of the occipital foramen toward the vertex is outlined in a lighter colour than the surrounding portions of the capsule; in this detail the larvae resemble *lanielensis* from which they are, however, at once distinguished by the black epiproct in *swainei.*

Thorax. Creamy yellow to light olive-green with the following marks: an olive-green, grayish or almost black subdorsal stripe running from Annulet *C* of the prothorax back to the abdomen; on the meso- and metathorax a supraspiracular dark spot on the base of *B*, *C*, and the dorsal portion of the postspiracular area; neck plates and sclerotized portions of legs black. The supraspiracular spots are often obsolescent and sometimes absent.

Abdomen. General colour similar to or lighter than that of the thorax; subdorsal stripe and supraspiracular row of spots continuous with those on thorax, the latter usually obsolescent at least on the middle segments. On the abdomen these spots when present occupy the bases of *B*, C^1, and C^2, sometimes extending to the dorsal portions of the spiracular and postspiracular areas. The epiproct bears a black patch separated into two portions by a wide and distinct median line.

Body coloration in this species is variable, especially in regard to the presence or absence of the supradorsal spots. In general, larvae with darker heads have a more greenish body colour and darker and more numerous spots. Larvae with black heads appear to be usually, if not always, males, but not all male larvae have black heads.

Food Plants. This species is known with certainty only from jack pine; one or two doubtful records of occurrence on red pine need to be authenticated by a competent observer.

Oviposition and Seasonal History. This species has a most characteristic pattern; the eggs are laid in pairs, one in each of the two needles which arise from a common base. The eggs are generally so spaced that each member of the pair occupies the same relative position on the needle (Fig. 52). In the field, they are laid only in new foliage, when the needles are about half grown. The full complement is generally laid on a single twig and the larvae are very gregarious. They are on the trees from July until October and pass the winter in the cocoon.

Parthenogenesis. Only males have been secured from the eggs of virgin females.

Summary

In Eastern Canada various species of sawflies belonging to the genus *Neodiprion* have been causing damage to the white, red, and jack pine through eating the needles. These sawflies belong to at least 12 species, some of which are rare and two of which are new to science. Keys and descriptions are provided for the recognition of the adult females and mature larvae. The sawflies are separable from each other also by their habits, notably those of oviposition, feeding, and overwintering.

Acknowledgments

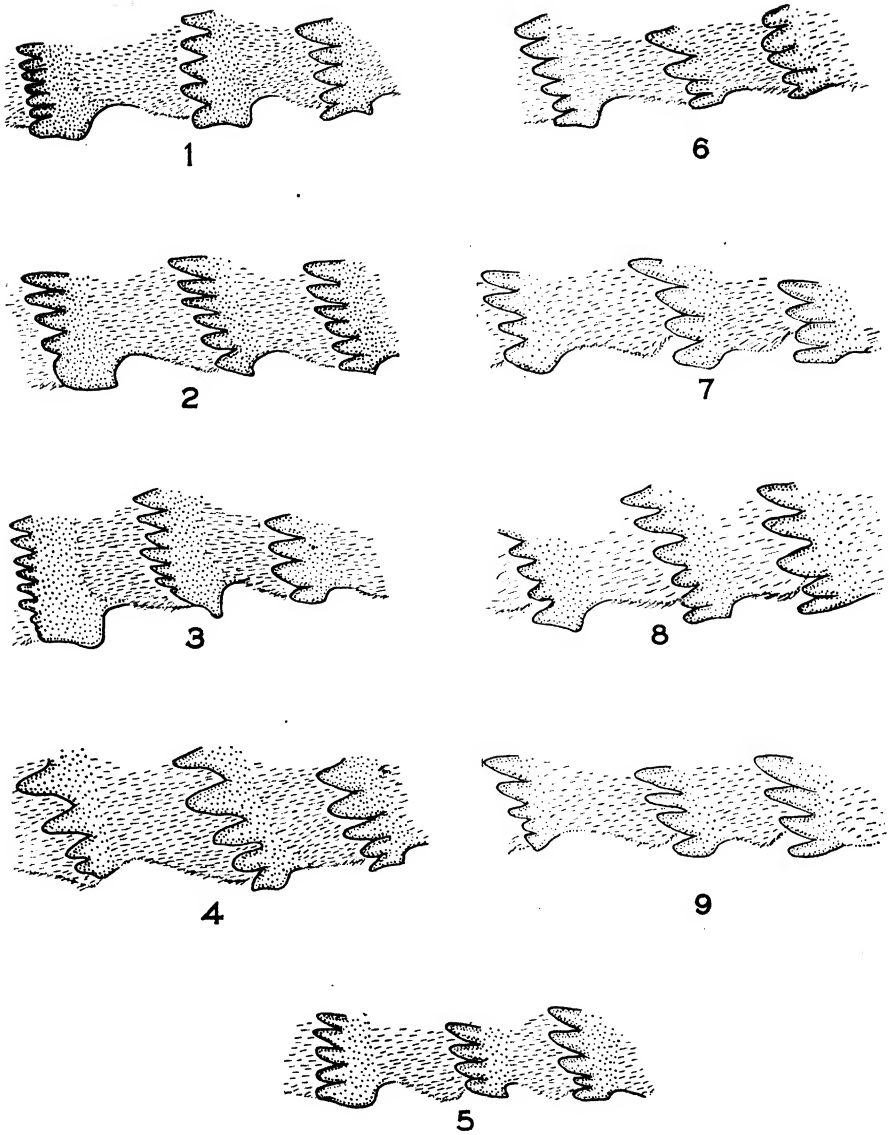
The writers wish to express their indebtedness to Dr. A. W. A. Brown and the staff of the Forest Insect Survey, who have aided in this work by supplying living material for rearing, pinned specimens, and data on distribution and food plants; to Dr. C. F. W. Muesebeck of the United States Department of Agriculture for supplying a pair of paratypes of *banksianae* and data in connection with certain types; to Mr. H. A. Richmond of the Entomological Laboratory at Winnipeg for supplying living larvae of one species; and to Miss Margaret MacKay of the Forest Insect Division for preparing figures of larval structures and egg scar patterns, with the exception of Figs. 1 to 15 which were drawn by O. Peck.

References

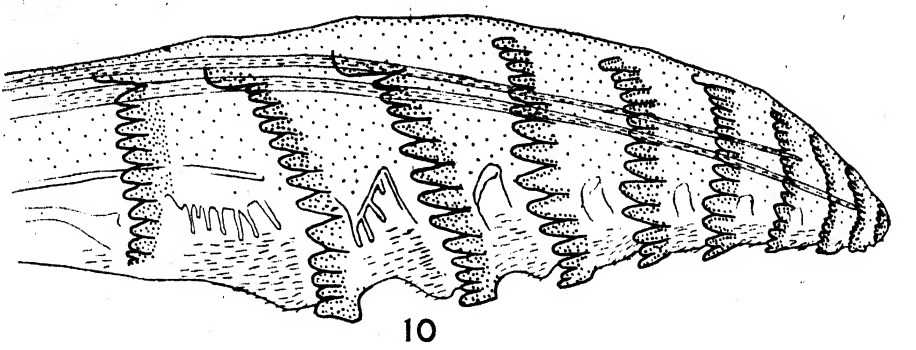
1. BENSON, R. B. On the genera of the Diprionidae (Hymenoptera Symphyta). Bull. Entomol. Research, 30 : 339-342. 1939.
2. BIRD, R. D. Notes on the fir sawfly, *Neodiprion abietis* Harr. Ann. Rept. Entomol. Soc. Ontario, 60 : 76-82. 1929.
3. BURKE, H. E. Two destructive defoliators of lodgepole pine in the Yellowstone National Park. U.S. Dept. Agr. Circ. 224. 1932.
4. CAN. DEPT. INTERIOR, Forest Service Bull. 61. Native trees of Canada. Rev. ed. 1933.
5. CRESSON, E. T. Description of new North American Hymenoptera in the collection of the American Entomological Society. Trans. Am. Entomol. Soc. 8 : 1-52. 1880.
6. DUNN, M. B. The jack pine sawfly. Can. Dept. Agr., Div. Forest Insects, Spec. Circ. 1931.
7. DYAR, H. G. A new sawfly. Proc. Entomol. Soc. Wash. 4 : 262-263. 1899.
8. FITCH, H. Fourth Report on the noxious and other insects of the State of New York. 1859.

9. GRAHAM, S. A. Two dangerous defoliators of jack pine. J. Econ. Entomol. 18 : 337-345. 1925.
10. HARRIS, T. W. A report on the insects of Massachusetts injurious to vegetation. Folsom, Wells and Thurston, Cambridge, Mass. 1841.
11. LEACH, W. E. The zoological miscellany; being descriptions of new or interesting animals. Vol. 3. Nodder and Son, London. 1817.
12. MIDDLETON, W. Leconte's sawfly, an enemy of young pines. J. Agr. Research, 20 : 741-760. 1921.
13. MIDDLETON, W. Two new species of sawflies of the subgenus *Neodiprion*. Proc. Entomol. Soc. Wash. 33 : 171-176. 1931.
14. MIDDLETON, W. Five new sawflies of the genus *Neodiprion* Rohwer. Can. Entomol. 65 : 77-84. 1933.
15. NORTON, E. *In* A. S. Packard's Guide to the study of insects and a treatise on those injurious and beneficial to crops: for the use of colleges, farm-schools and agriculturists. Salem. 1869.
16. NORTON, E. Catalogue of the described Tenthredinidae and Uroceridae of North America. Trans. Am. Entomol. Soc. 2 : 321-368. 1869.
17. ROHWER, S. A. New western Tenthredinidae. J. New York Entomol. Soc. 16 : 103-114. 1908.
18. ROHWER, S. A. New sawflies of the subfamily Diprioninae (Hym.). Proc. Entomol. Soc. Wash. 20 : 79-90. 1918.
19. ROHWER, S. A. Notes on sawflies, with description of new genera and species. Proc. U.S. Natl. Museum, 59 : 83-109. 1922.
20. ROHWER, S. A. Description of a new sawfly injurious to jack pine. Proc. Entomol. Soc. Wash. 27 : 115-116. 1925.
21. ROSS, H. H. The sawfly genus *Empria* in North America (Hymenoptera, Tenthredinidae). Pan-Pacific Entomol. 12 : 172-178. 1936.
22. ROSS, H. H. A generic classification of the nearctic sawflies (Hymenoptera, Symphyta). Illinois Biol. Monogr. 15, No. 2. 1937.
23. RUGGLES, A. G. Report of the State Entomologist of Minnesota, 19 : 8. 1922.
24. SAY, T. Appendix to Keating's (W. H.) Narrative of an expedition to the source of St. Peter's River, Lake Winnepeek, Lake of the Woods 1822, under the command of Stephen H. Long. U.S.T.E. 2 : 315. 1824. (Reprinted by Le Conte, J. L. The complete writings of Thomas Say on the entomology of North America, I : 210-211. New York. 1859.)
25. SCHEDL, K. Notes on jack pine sawflies in Northern Ontario. Ann. Rept. Entomol. Soc. Ontario, 61 : 75-79. 1931.
26. SCHEDL, K. Statistische Untersuchungen über die Kopfkapselbreiten bei Blattwespen. Z. angew. Entomol. 20 : 449-460. 1933.
27. SCHEDL, K. Zwei neue Blattwespen aus Kanada (Hym., Tenthr.). Mitt. deut. entomol. Ges. 6 : 39-44. 1935.
28. SCHEDL, K. Quantitative Freilandstudien an Blattwespen der *Pinus banksiana* mit besonderer Berücksichtigung der Methodik. Z. angew. Entomol. 24 : 25-70, 181-215. 1937.
29. YUASA, H. A classification of the larvae of the Tenthredinoidea. Illinois Biol. Monogr. 7, No. 4. 1922.

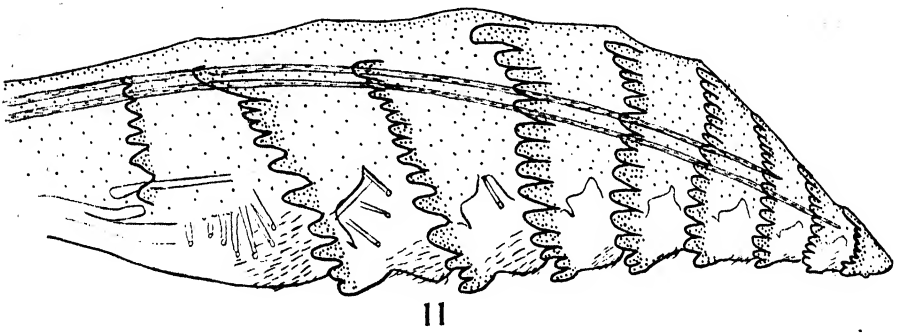
Note: Figs. 1 to 53 will be found on pages 139–144.



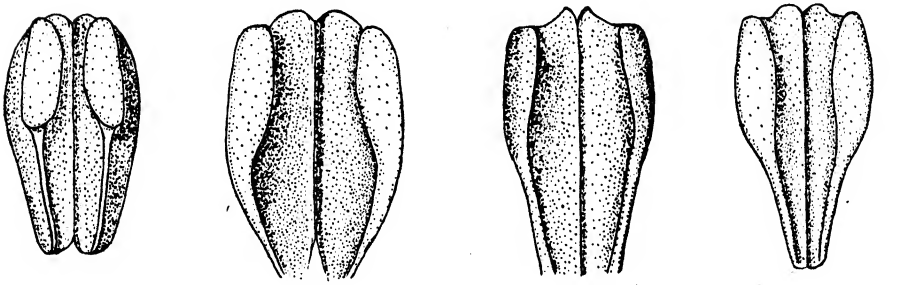
FIGS. 1 – 9. *First, second, and third ventral lobes of lancet.* 1. *Neodiprion lecontei (Fitch).* 2. *N. maurus Roh.* 3. *N. nanulus Schedl.* 4. *N. nigroscutum Midd.* 5. *N. banksianae Roh.* 6. *N. pinetum (Nort.).* 7. *N. rugifrons Midd.* 8. *N. swainei Midd.* 9. *N. ferrugineus Midd.*



10

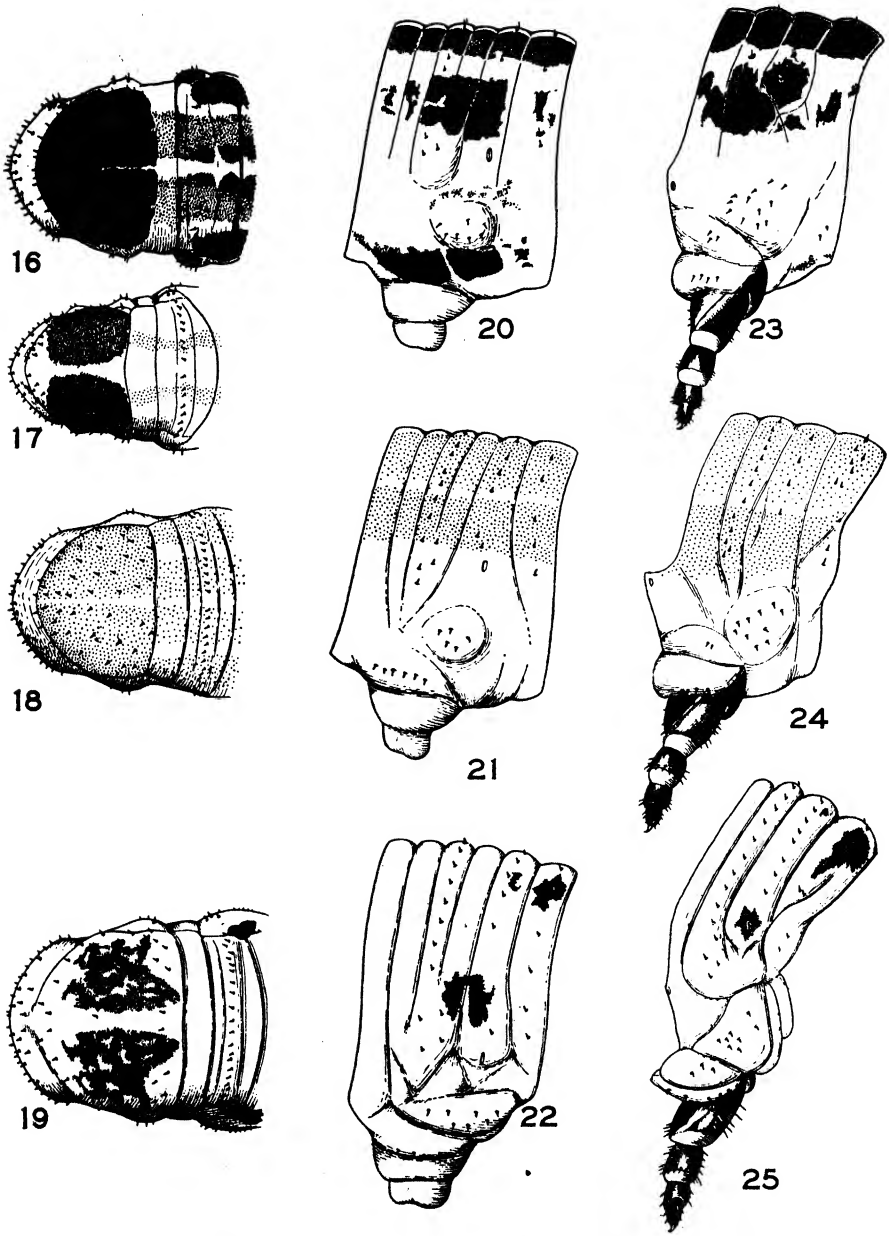


11

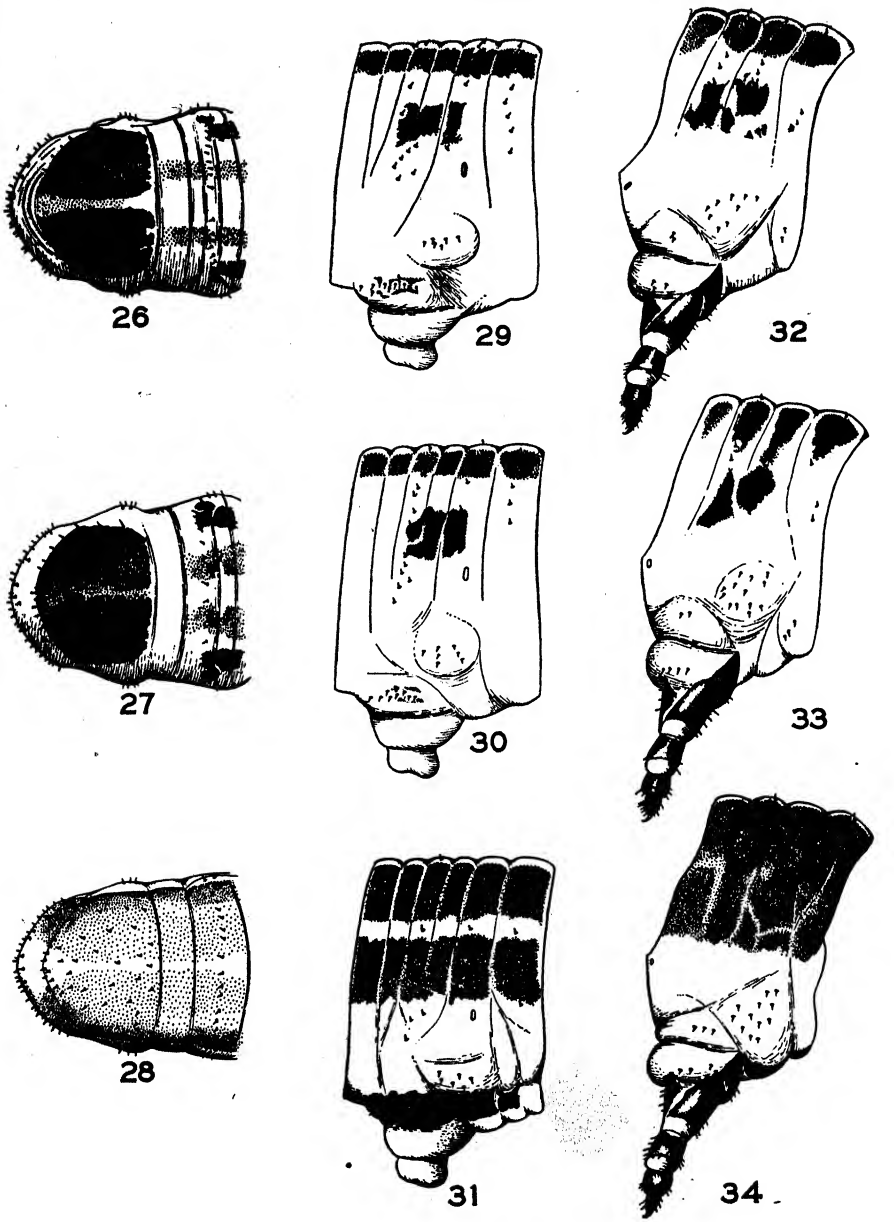


12 13 14 15

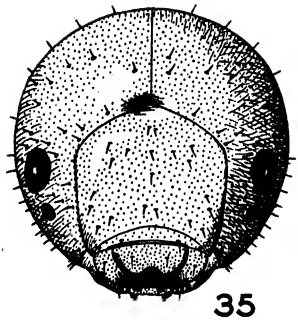
FIGS. 10, 11. *Lateral view of lancet.* 10. *Neodiprion lanielensis n. sp.* 11. *N. flemingi n. sp.* FIGS. 12 – 15. *Posterior view of scopa.* 12. *N. lanielensis n. sp.* 13. *N. flemingi n. sp.* 14. *N. swainei Midd.* 15. *N. banksianae Roh.*

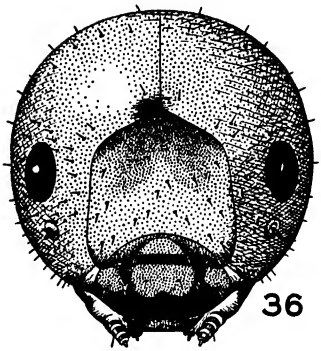


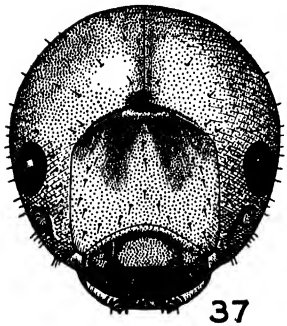
FIGS. 16 – 25. *Larval colour pattern. Supra-anal area:*—16. *Neodiprion rugifrons Midd.* 17. *N. swainei Midd.* 18. *N. flemingi n. sp.* 19. *N. lecontei (Fitch). Typical abdominal segment:*— 20. *N. rugifrons Midd.* 21. *N. flemingi n. sp.* 22. *N. lecontei (Fitch). Mesothoracic segment:*— 23. *N. rugifrons Midd.* 24. *N. flemingi n. sp.* 25. *N. lecontei (Fitch).*

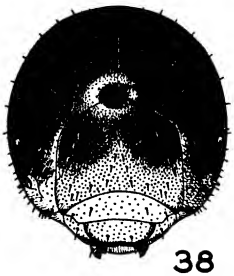


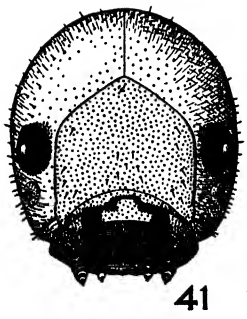
FIGS. 26 – 34. *Larval colour pattern. Supra-anal area:*— 26. *Neodiprion nanulus Schedl.* 27. *N. maurus Roh.* 28. *N. lanielensis n. sp. Typical abdominal segment:*— 29. *N. nanulus Schedl.* 30. *N. maurus Roh.* 31. *N. lanielensis n. sp. Mesothoracic segment:*— 32. *N. nanulus Schedl.* 33. *N. maurus Roh.* 34. *N. lanielensis n. sp.*

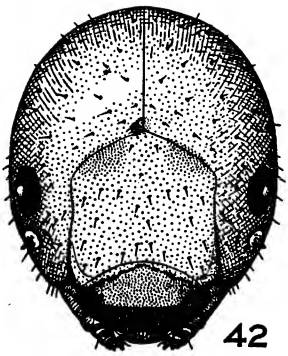


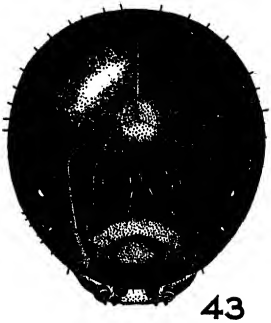




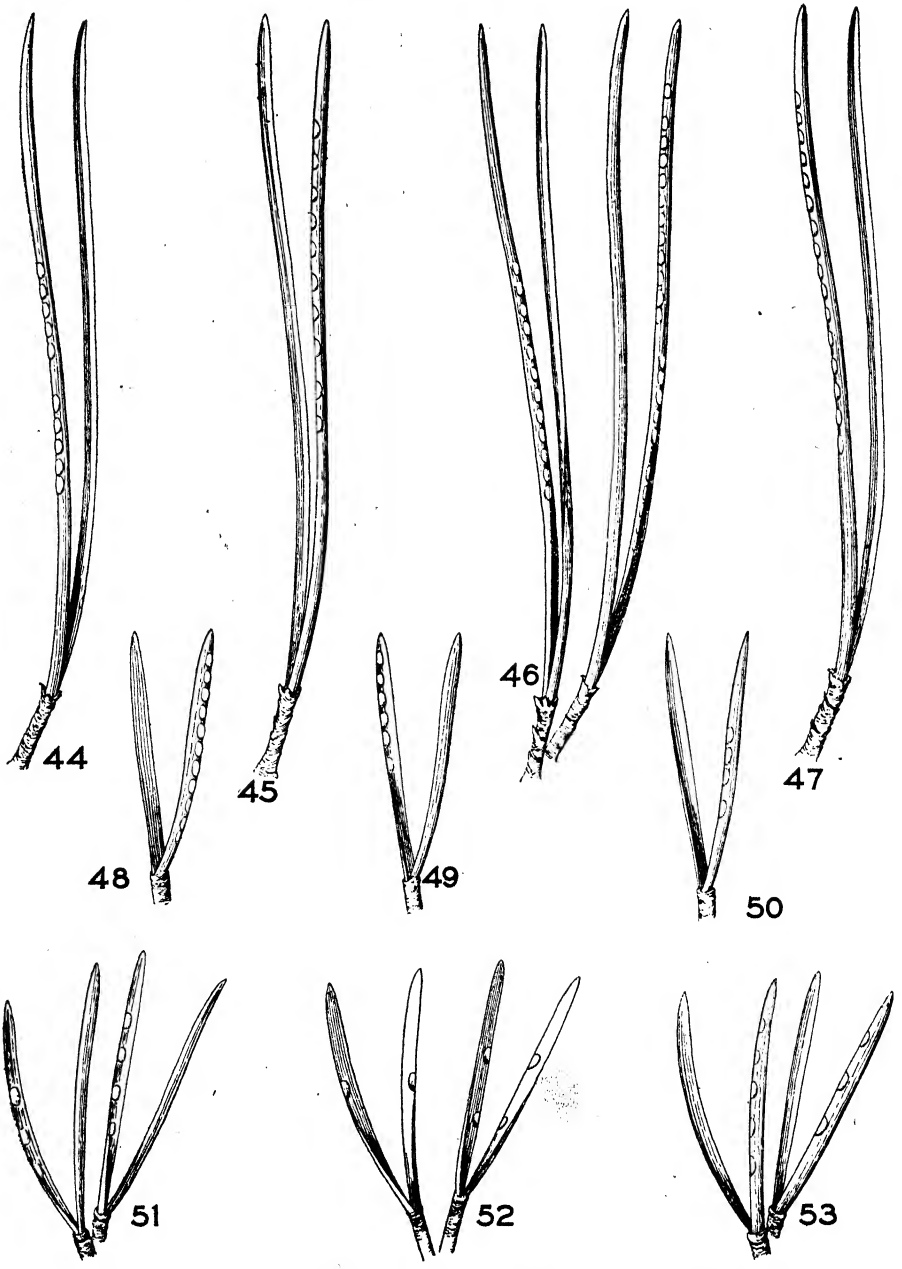








FIGS. 35 – 43. *Colour patterns of larval head capsules.* 35 – 37. *Variations in brown-headed forms of Neodiprion swainei Midd.* 38. *Black-headed form of N. swainei Midd.* 39. *N. lanielensis n. sp. (dorsal view).* 40. *N. lanielensis n. sp.* 41. *N. rugifrons Midd., II stage.* 42. *N. rugifrons Midd., V stage* ♀. 43. *N. flemingi n. sp.*



FIGS. 44 – 53. *Egg scar patterns. In red pine needles:—* 44. *Neodiprion lanielensis n. sp.* 45. *N. nanulus Schedl.* 46. *N. leconteí (Fitch).* 47. *N. flemingi n. sp. In jack pine needles:—* 48. *N. rugifrons Midd.* 49. *N. maurus Roh.* 50. *N. lanielensis n. sp.* 51. *N. banksianae Roh.* 52. *N. swainei Midd.* 53. *N. nanulus Schedl.*

PROTEOCEPHALUS PARALLACTICUS, A NEW SPECIES OF TAPEWORM FROM LAKE TROUT, *CRISTIVOMER NAMAYCUSH*[1]

By D. A. MacLulich[2]

Abstract

A new species of tapeworm, *Proteocephalus parallacticus*, collected from lake trout, *Cristivomer namaycush* (Walbaum), in Algonquin Provincial Park, Ont., is described.

A proteocephalid tapeworm, abundant in trout in Algonquin Provincial Park and collected from them during a survey of the parasites of these fish (9), did not correspond to any published descriptions (1-8, 10-14) and is therefore described as a new species. The field work was carried out under the auspices of the Ontario Fisheries Research Laboratory while the author was on the staff of Algonquin Park, and facilities for the laboratory work were provided by the Ontario Research Foundation by the courtesy of Dr. S. Hadwen and the Royal Ontario Museum of Zoology by Dr. J. R. Dymond, Director. Dr. George R. LaRue has kindly examined specimens and the description and has agreed that this form is not *Proteocephalus pusillus* (Ward) nor any other form known to him.

Description

The species described agrees with characters of the family Proteocephalidae, genus *Proteocephalus* Weinland and subgenus *Teleostotaenia* Woodland (14).

Strobila

The strobila vary in length, commonly up to 30 mm. (one specimen 82 mm.); the width may reach 1.5 mm. The dimensions of the *proglottids* at 7.5 mm. from the anterior of the worm = 0.38 mm. in length × 0.80 mm. in width; at 30 mm. from anterior = 1.14 × 1.06 mm.; at 80 mm. from anterior = 1.32 × 1.37 mm. The proglottids may show no external evidence of segmentation or may be rounded with a sharp constriction between each.

Neck

The unsegmented part behind the suckers to the first segmentation groove varies from 1.7 to 7.5 mm. in length, average = 3.5 mm.

Scolex

The scolex is square or oblong in anterior view, 0.2 to 0.6 mm. wide. There are four functional cup-shaped acetabula or *suckers* which face usually forward and slightly outward; an inconspicuous, smaller, non-functional, apical fifth sucker is present in mature worms. The functional suckers are

[1] *Manuscript received in original form May 12, 1942, and as revised, February 1, 1943. Contribution from the Ontario Fisheries Research Laboratory, Department of Zoology, University of Toronto, Toronto, Ont.*

[2] *At the time, on the ranger staff of Algonquin Provincial Park, Ont. Now in the Navigation Branch of the Royal Canadian Air Force.*

circular in outline, the diameter of the opening being about 0.1 mm. The fifth sucker is rarely seen in living worms; it was not cup-shaped in any of the material examined but conformed to the contour of the apex of the scolex.

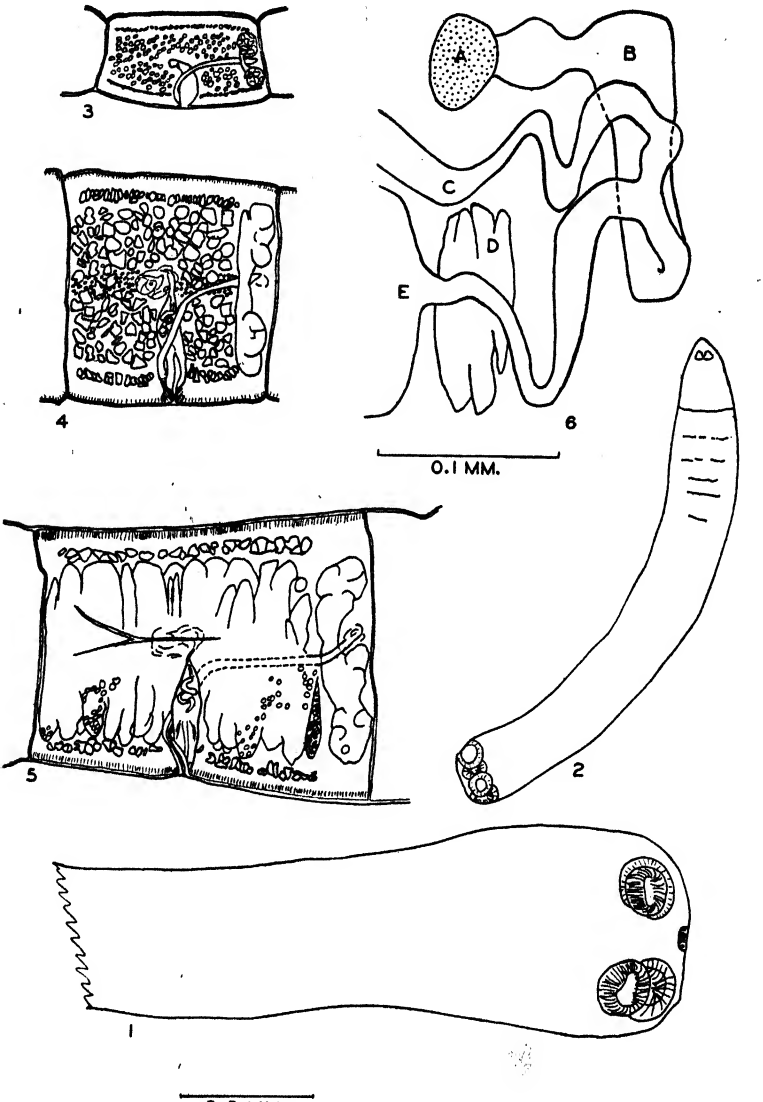


FIG. 1. *Immature worm.* FIG. 2. *Scolex of mature worm.* FIG. 3. *Young proglottid, ventral view.* FIG. 4. *Mature proglottid, ventral view.* FIG. 5. *Ripe proglottid, dorsal view.* FIG. 6. *Connections of female genital system, built up from serial sections. Orientation: left = anterior, top = ventral. (A) = bridge of ovary, (B) = oviduct, (C) = vagina, (D) = oötype, (E) = uterus.*

In stained and cleared mounts, this sucker is seen as an oval muscle mass with a basement membrane, the whole mass being 0.06 mm. in diameter, 0.03 mm. in thickness. The surface of the scolex between the acetabula varies from smooth to depressed, often with a pair of opposed grooves, resulting in a flattened cross section.

Genital Pores

The genital pores occur laterally at the midpoint of the segment, alternating irregularly from side to side; there is no papilla and the pore is usually in a depression.

Male Reproductive Organs

The *cirrus sac* is an elongate, pyriform pouch extending across more than one-third the width of the proglottid and nearly to the middle. The dimensions are 0.39 to 0.42 mm. by 0.11 to 0.14 mm. The *cirrus* is straight and cylindrical when invaginated and a slender cone with a blunt end when everted. The *ductus ejaculatorius* forms one to two, or rarely three, irregular coils within the inner half of the cirrus sac. The *vas deferens* consists of a small mass of coils in the centre of the proglottid, extending slightly anterior to the cirrus pouch, visibly connected to the ductus ejaculatorius but the tubules from the testes are rarely visible. The *testes* are somewhat flattened ovals in shape, in one irregular layer, a few overlapping each other. They vary in their three dimensions from 0.100 deep $\times$ 0.081 $\times$ 0.063 mm., to 0.120 $\times$ 0.110 $\times$ 0.076 mm. and average 0.11 $\times$ 0.09 $\times$ 0.07 mm. The number per segment varies from 45 to 92, the average number for 22 specimens being 71 testes per segment.

Female Reproductive Organs

The ovary is bilobed, the lobes quadrate, connected anteriorly by a slender bridge, becoming more irregular and lobulated in older proglottids. The *vitellaria* form a narrow line of small follicles down each side, inside the muscle zone, interrupted at the cirrus sac. They range in diameter from 0.045 to 0.065 mm. The *vagina* opens to the exterior dorsal to the cirrus sac and close beside it, within the depression or pit. The sphincter vaginae are insignificant and rarely seen. The vagina crosses the anterior portion of the cirrus sac obliquely—then passes ventrally across the part of the cirrus sac between one-half and three-fourths of its length from the exterior. The receptaculum seminalis, if present, is small; it may be entirely lacking. The vagina extends between the two halves to the rear of the ovary where it joins the *oviduct* which comes directly from near the bridge of the ovary. The oviduct leads forward again with a small amount of coiling to a rosette-like oötype and thence the *uterus* extends forward dorsal to the testes. In younger proglottids the uterus appears as a central column which in other segments is

hollow and in ripe segments is multilobed and fills much of the proglottid. The number of lateral pouches on each side of a segment varies from seven on the cirrus side and nine on the other to 10 and 12 and 13 and 15. The *eggs* are released by a splitting open of the body wall and uterus. The intrauterine eggs measured 0.012 to 0.015 mm. in diameter.

Hosts

Specimens were found in the intestine of lake trout, *Cristivomer namaycush* (Walbaum), in speckled trout, *Salvelinus fontinalis* (Mitchill), and in brown trout, *Salmo fario* Linnaeus.

Material

The type specimen was collected from the intestine of a lake trout, *C. namaycush*, No. 96, in Lake Opeongo, Algonquin Provincial Park, Ont., Canada, on July 28, 1939. Slides and specimens preserved in alcohol are deposited in the Royal Ontario Museum of Zoology and two slides have been sent to the United States National Museum. Many other specimens from several hundred lake and speckled trout from various lakes in Algonquin Park were examined.

Discussion

The species name, *parallacticus*, refers to the manner in which the vagina crosses over the cirrus sac. The form described is distinguished from *Proteocephalus pusillus*, the only species with which it is liable to be confused, by the crossing of the vagina around and under the cirrus sac, which *never* occurs in *P. pusillus*. This is a prominent feature in whole mounts. Other differences, such as the larger size and number of some of the parts, are not of such positive diagnostic value as the above character.

References

1. BANGHAM, R. V. A study of the cestode parasites of black bass in Ohio, with special reference to their life history and distribution. Ohio J. Sci. 25 : 255-270. 1925.
2. CROSS, S. X. A study of the fish parasite relationships in the Trout Lake region of Wisconsin. Trans. Wisconsin Acad. Sci. 31 : 439-456. 1938.
3. FAUST, E. C. Two new Proteocephalidae. J. Parasitol. 6 : 79-83. 1920.
4. HOFF, E. C. and HOFF, H. E. Proteocephalus pugetensis, a new tapeworm from a stickleback. Trans. Am. Micr. Soc. 48 : 54-61. 1929.
5. HUNTER, G. W. and BANGHAM, R. V. Studies on the fish parasites of Lake Erie. II. New cestoda and nematoda. J. Parasitol. 19 : 304-311. 1933.
6. LARUE, G. R. On the morphology and development of a new cestode of the genus Proteocephalus Weinland. Univ. Nebraska Studies, Zool. Lab. 95 : 17-49. 1909.
7. LARUE, G. R. A revision of the cestode family Proteocephalidae. Illinois Biol. Monogr. 1. 1914.
8. LARUE, G. R. A new species of tapeworm of the genus Proteocephalus from the perch and the rock bass. Occasional Papers Museum Zool. Univ. Michigan, No. 67 : 1-10. 1919.
9. MACLULICH, D. A. Parasites of trout in Algonquin Provincial Park, Ontario. Can. J. Research, D. In preparation.

10. MEGGITT, F. J. Remarks on the cestode families Monticellidae and Ichthyotaeniidae. Ann. Trop. Med. 21 : 69-87. 1927.
11. WARD, H. B. Internal parasites of the Sebago salmon. Bull. U.S. Bur. Fisheries, 28 : 1154-1194, Document 713. 1910.
12. WARDLE, R. A. The cestoda of Canadian fishes. II. The Hudson Bay drainage system. Contrib. Can. Biol. Fisheries (n.s.), 7 (30) : 377-403. 1932.
13. WOODLAND, W. N. F. On a new Bothriocephalus and a new genus of Proteocephalidae from Indian fresh-water fishes. Parasitology, 16 : 441-451. 1924.
14. WOODLAND, W. N. F. On three new Proteocephalids (cestoda) and a revision of the genera of the family. Parasitology, 17 : 370-394. 1925.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. JUNE, 1943 NUMBER 6

OBSERVATIONS ON THE FATE OF PHENOTHIAZINE IN DOMESTIC ANIMALS[1]

BY H. B. COLLIER[2], D. E. ALLEN[3], AND W. E. SWALES[4]

Abstract

The investigation of the fate of phenothiazine in sheep, horses, dogs, rabbits, and pigs has been based upon the identification of the urinary excretion products, which was facilitated by chromatographic analysis and observation of absorption spectra.

In the sheep the drug is oxidized in the rumen and is excreted as leucophenothiazone conjugated with sulphuric acid. Horses and dogs also excrete leucophenothiazone, whereas rabbits excrete mainly leucothionol, all in conjugated form. The urine of pigs, after acidification, contains free phenothiazine, with smaller amounts of thionol and phenothiazone. These observations on the fate of phenothiazine indicate no fundamental difference between herbivorous and other animals.

Some aspects of the fate of phenothiazine in sheep have been discussed in an earlier contribution from this laboratory (5), where it was demonstrated that the drug is excreted largely in the form of leucophenothiazone, conjugated with sulphuric acid. The present paper includes more detailed study on the sheep, involving the use of chromatographic analysis and spectroscopic observation in the identification of phenothiazine derivatives. Less detailed observations on several other species of domestic animals are also recorded.

Experimental

A. SHEEP

Synthesis of Conjugate

In an endeavour to confirm the structure of the conjugate excreted in the urine of sheep (5), leucophenothiazone sulphuric acid was synthesized by the method of Burkhardt and Lapworth (4). Leucophenothiazone prepared by the method of Bernthsen (3) was treated with chlorosulphonic acid under the conditions prescribed. The crude conjugate was recrystallized from water and its properties were compared with those of the natural conjugate isolated from sheep urine. As indicated by the following analysis, the product was

[1] *Manuscript received in original form November 2, 1942, and as revised, March 22, 1943. Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.*

[2] *At the time, Lecturer, Institute of Parasitology. At present, Assistant Professor, Department of Biochemistry, Dalhousie University, Halifax, N.S.*

[3] *Research Assistant.*

[4] *Associate Animal Pathologist, Science Service, Dominion Department of Agriculture.*

evidently not pure compared with the theoretical values for potassium leucophenothiazone sulphate, $C_{12}H_8NSOSO_3K$.

Potassium	9.2% found,	11.7% calculated
SO_3	19.9% found,	24.0% calculated
Ash	22.0% found,	26.1% calculated

The following melting points were observed (not corrected):—

Synthetic conjugate	215 – 220° C. (decomp.)
Sheep conjugate	241 – 242° C. (decomp.)
Mixed conjugates	230 – 235° C. (decomp.)

Spectrometry

To aid in the identification of the phenothiazine derivatives in sheep urine, the solutions were examined visually by means of a spectrometer with a calibrated wave-length scale. Pure *phenothiazone* in neutral aqueous solution showed no absorption bands. In strongly acid solution, after heating or standing, there was observed a sharp band at 555 mμ. This has been shown by Pummerer, Eckert, and Gassner (16) and by Granick, Michaelis, and Schubert (9) to be the absorption band of the semiquinone form of phenothiazone. *Thionol* in neutral or acid solution was cherry red, and showed no absorption bands. In alkaline solution, on the other hand, it was an intense blue-purple colour, giving a strong broad band at 590–600 mμ; this presumably is the spectrum of the phenolate ion, the possibility of free radical formation in alkaline solution not having been investigated.

According to Smiles (1, 2, 10), *sulphonium salts* are obtained when phenothiazine sulphoxide is dissolved in acid, or when phenothiazine itself is heated in strong acid. The solutions are orange-pink in colour and give a typical series of absorption bands: the spectrum has been shown by Kehrmann (12) and by Michaelis and co-workers (15) to be that of the semiquinone of phenothiazine, which is very stable in acid solution. With a small spectrometer it was possible to observe the three bands of longer wave-length, at 518, 497, and 480 mμ.

Synthetic conjugate, when treated with hydrochloric acid and ferric chloride, gave a red solution and a strong absorption band at 555 mμ indicating phenothiazone. A solution in 10 *N* sulphuric acid was brown-red in colour, with bands at 526 and 505 mμ; when the solution was heated or allowed to stand, the purple colour and absorption band of phenothiazone appeared. The sheep conjugate was found to exhibit identical properties. Also, when the urine of treated sheep was added to cold 10 *N* sulphuric acid, with a little ceric sulphate as oxidizing agent, the same brown colour and band at 526 mμ were observed. Phenothiazone was detected only after standing or heating. The similarity between the sheep conjugate and the synthetic conjugate may be taken as further evidence that the structure assigned is the correct one. The absorption spectrum of the conjugate is of the phenothiazine semiquinone type, except that the bands are shifted 8 mμ toward the red. It is possible that oxidation prior to hydrolysis may account for this type of spectrum.

Chromatographic Analysis

Lipson (13, 14) has demonstrated the value of chromatographic analysis in separating and identifying the oxidation products of phenothiazine. He found that, using chloroform solution and an alumina column, thionol is strongly adsorbed, whereas phenothiazone passes through the column unadsorbed. The writers' experiments have confirmed these observations; in addition it was found that the sulphonium salts are even more strongly adsorbed than thionol, forming a brown band at the top of the column.

Urine of treated sheep was acidified with hydrochloric acid and was extracted with chloroform*. When the extract was passed through the column it was observed that the pigment was almost wholly phenothiazone containing only a trace of thionol.

Oxidation and Absorption of Phenothiazine

A sheep was given 100 gm. of phenothiazine in the form of compressed tablets. It was killed five hours after dosing and the contents of the gastro-intestinal tract were immediately examined for the drug and its oxidation products. The rumen was found to contain 58 gm. of unchanged phenothiazine, determined by extraction with hot alcohol and colorimetric estimation as previously described (5); phenothiazone was also present to the extent of about 4 gm. The abomasum and small intestine contained appreciable amounts of phenothiazine and traces of phenothiazone; the contents of the large intestine showed only a small amount of phenothiazine. The bladder bile contained conjugate equivalent to 0.05% phenothiazone.

It was concluded from these observations that phenothiazine is rapidly oxidized in the rumen, possibly through the agency of bacterial action, enhanced by the alkaline reaction of the contents. Absorption may also take place in the rumen, since the traces of phenothiazone in the abomasum and small intestine appeared insufficient to account for the appearance of this derivative in the blood and urine within less than 30 min. after dosing (19). Conjugation probably takes place in the liver; the biliary excretion of derivatives may be compared with the similar finding of DeEds and Thomas (8) in rats, rabbits, and humans.

The biliary excretion may explain the presence of oxidation products in the intestinal tract and faeces. Although it was previously reported (5) that these are absent from faeces, a modified method served to reveal their presence, as indicated in Table I. The dried faeces were extracted with hot water; the extract after filtration was treated with hydrochloric acid and ferric

* *The following values have been obtained for chloroform/water partition coefficients of phenothiazine derivatives, the initial concentration of the aqueous phase being 5 mg. per 100 ml. in each case.*

Phenothiazone, in M/15 phosphate, pH 7	*500*
Thionol, in M/15 phosphate, pH 7	*0.7*
Thionol, in M/15 phosphate, pH 2	*5.2*
Sulphonium chloride (sulphoxide in N HCl)	*14*

n-Butanol was found to be excellent for extracting both phenothiazone and thionol, but it was unsuitable as a medium for chromatographic adsorption.

TABLE I

EXCRETION OF PHENOTHIAZINE BY SHEEP

Animals: yearling lambs, approximately 80 lb.
Dosage: pure phenothiazine in compressed tablets, standard formula (18).
Excretion: phenothiazine and phenothiazone expressed as phenothiazine, and as per cent of the total dose.

Description	Faeces			Urine, phenothiazone		Total excretion, %
	Phenothiazine, gm.	Phenothiazone, gm.	Total, %	Gm.	%	
Lamb No. 22, 10 gm. micronized phenothiazine	1.09	—	10.9	7.51	75.1	86
Lamb No. 14, 10 gm. micronized phenothiazine plus 0.2 gm. extra phenolphthalein	0.87	0.13	10.0	5.50	55.0	65
Lamb No. 14, 10 gm. micronized phenothiazine plus 1.0 gm. extra phenolphthalein	0.70	0.25	9.5	9.55	95.5	105
Lamb No. 20, 10 gm. micronized phenothiazine plus 10 gm. bentonite	0.90	0.35	12.5	8.71	87.1	100
Lamb No. 18, 10 gm. comml. phenothiazine	2.36	0.48	28.4	6.45	64.5	93
Lamb No. 18, 10 gm. comml. phenothiazine	3.02	0.55	35.7	6.11	61.1	97
Lamb No. 22, 10 gm. comml. phenothiazine	2.30	0.73	30.3	6.06	60.6	91
Lamb No. 22, 50 gm. comml. phenothiazine	20.6	0.95	43.1	22.9	45.8	89

chloride. Colorimetric readings were then made before and after reduction with stannous chloride, the difference being calculated as phenothiazone.

The quantitative analysis of urine was also modified in a similar manner. For the oxidation of the acid urine, ferric chloride was substituted for hydrogen peroxide, which brought about a slow bleaching. After the reading was noted, a blank reading was obtained by bleaching the pigment with a small crystal of stannous chloride; the difference was calculated as phenothiazone. In the sheep, these modified methods gave practically complete recovery of the original dose (Table I).

Effect of Particle Size upon Absorption Rate

In order to gain further information regarding rates of absorption of phenothiazine and their relationship to anthelmintic efficiency, a group of yearling sheep, each weighing approximately 80 lb., was treated with phenothiazine of different particle sizes. Micronized phenothiazine, in which about 80% of the particles were found to be less than four microns in diameter, was prepared in tablet form according to Swales (18); for purposes of comparison ordinary commercial phenothiazine, in which more than 80% of the particles were over four microns, was used after similar preparation in tablet form. The

results of these tests are summarized in Table I. It was concluded that the micronized drug is more completely oxidized and absorbed, only about 10% being excreted unchanged in the faeces, whereas from 28 to 36% of the commercial form was thus excreted when comparable doses were used.

Extra phenolphthalein was added to the formula in two experiments in order to test the effect of increased laxative action: the somewhat increased faecal output did not increase the proportion of micronized phenothiazine that remained unabsorbed in the faeces. Grinding the phenothiazine powder with bentonite in a mill for 12 hr., at the end of which time the mixture was deep red in colour, did not change the rate of absorption.

This work on the effect of particle size was not extended to critical anthelmintic tests because of the above finding that micronized phenothiazine was so rapidly absorbed and excreted in the urine.

Administration of Phenothiazone

If phenothiazone is the oxidation product in the sheep, it may also be the active anthelmintic substance. Accordingly, this derivative was administered to sheep. In the first test the product, prepared by the method of Pummerer and Gassner (17), was contaminated with large amounts of iron compounds; the 42 gm. of crude substance administered contained only 23 gm. of pure phenothiazone. The sheep died eight hours after administration of this dose; apparently death was due to poisoning from the phenothiazone or the impurities. At this time, only 5 gm., as pure phenothiazone, remained in the rumen, indicating extremely rapid absorption; only traces of the pigment were detected in the remainder of the gastrointestinal tract. Both the blood and the bladder bile were found to contain conjugated phenothiazone, in concentrations of 0.07% and 0.4% respectively. The excretory product in the urine consisted largely of a conjugate of phenothiazone (or of its leuco form), reaching a concentration of 2% before death.

In another experiment the crude phenothiazone was recrystallized from alcohol and the red crystals resulting from this treatment assayed practically 100% pure. A compressed tablet containing 5 gm. of this pure compound was administered to a sheep. No ill effects were observed clinically.

Free phenothiazone was detected in the faeces; this compound was found in the urine, both free and conjugated, but no leucophenothiazone was detected. The data from quantitative analyses of the excreta are presented in Table II. A part of the urine was cooled; this resulted in the crystallization of a conjugate which, when dissolved in 10 *N* sulphuric acid and treated with ceric sulphate, gave absorption bands at 526 and 505 mμ, typical of the conjugate excreted after administration of phenothiazine. This is further evidence that the conjugate recovered from sheep is a conjugated form of phenothiazone.

Although the animal used in this test was infected with *Haemonchus contortus* and other nematode parasites that are easily removed by medication with phenothiazine, no nematodes were expelled and the pretreatment count of 6000 eggs per gram of faeces remained unchanged after the above dose of phenothiazone.

TABLE II

EXCRETION OF PHENOTHIAZONE BY A SHEEP

Animal: 16 month old wether, wt. 90 lb.
Dosage: 5 gm. of recrystallized phenothiazone.
Excretion: phenothiazone expressed as such.

Time after dosing, hr.	Volume, ml.	Dry weight, gm.	Phenothiazone		
			%	Gm.	Gm./hr.
Urine					
0 – ½	26		0.091	0.02	0.047
½ – 5	200		0.435	0.87	0.193
5 – 8	187		0.531	1.00	0.333
8 – 23	370		0.594	2.20	0.147
23 – 28	96		0.378	0.36	0.072
28 – 32	9		0.560	0.05	0.012
32 – 47	228		0.175	0.40	0.027
47 – 55½	100		0.086	0.09	0.011
55½ – 71	179		0.066	0.12	0.008
Total				5.11	
Faeces					
0 – 8		45	0.004	0.002	0.0002
8 – 23		246	0.017	0.042	0.0028
23 – 32		90	0.043	0.039	0.0043
32 – 47		86	0.026	0.022	0.0015
47 – 56		103	0.005	0.005	0.0005
56 – 71		140	0.001	0.001	0.0001
Total				0.111	

NOTE: *Total recovery: 5.22 gm. = 104%.*

B. HORSES

Identification of Excretory Products

When horses were treated with phenothiazine the derivatives in the blood and urine were found to be qualitatively the same as in sheep. Crystals of a conjugate of leucophenothiazone were isolated from horse urine on cooling, and these showed the same properties as the "sheep conjugate"; the "horse conjugate", in 10 *N* sulphuric acid, exhibited the same absorption spectrum, having bands at 526 and 505 mμ.

When chromatographic adsorption was applied to the horse urine, the major portion of the red pigment was shown to be phenothiazone. A trace of thionol was adsorbed on the alumina, and in addition there were several brown bands, which were probably those of normal urinary pigments.

It was concluded that, qualitatively, the fate of phenothiazine in the horse follows the same path as in the sheep. Quantitatively, however, the writers were never able to achieve more than 50% recovery of the original dose (20). Whether this is due to incomplete excretion of the drug, to inadequate methods, or to the presence of unidentified excretion products, is not known at present.

Post-mortem Examination

A horse was killed two days after being given 80 gm. of phenothiazine, and the various organs were examined for derivatives, but the results were largely negative. The tissues of the liver, spleen, kidney, and the bone marrow were extracted with hydrochloric acid and treated with ferric chloride, but no colour was apparent. It was evident, however, that derivatives were present, because the surfaces of the liver, spleen, and kidneys that were exposed to the air turned a dark red, in contrast to the non-exposed surfaces, which retained normal coloration. It is probable that the phenothiazine derivatives combine with tissue proteins, and no suitable method of extraction or of detection has been evolved.

C. Dogs

Preliminary observations on dogs treated with phenothiazine have indicated that the excretory products are similar to those of sheep and horses. The red pigment of acidified urine was identified as phenothiazone, through its chromatographic and spectroscopic behaviour.

D. Rabbits

The acidified urine of rabbits dosed with phenothiazine showed an absorption spectrum having bands at 522 and 497 mμ. This spectrum is of the phenothiazine semiquinone type, but is not identical with it, or with the spectrum of the sheep conjugate. The phenothiazone band at 555 mμ was also observed. A chromatogram revealed a large amount of thionol, as reported by DeEds (7), and a smaller concentration of phenothiazone.

E. Pigs

The results obtained with treated pigs were markedly different from the findings reported for the other species. No conjugate crystallized out on cooling the urine, and tests for conjugates of sulphuric, glucuronic, or acetic acids were negative. The conjugates of sheep and of human urine may be extracted with *n*-butanol (6), but this procedure when applied to pig urine gave a negative result.

Urine acidified with hydrochloric acid was orange in colour, and showed a strong absorption band at 518 mμ, identical with that of the phenothiazine semiquinone. No phenothiazone was detected spectroscopically, even after heating. When the acid urine was made alkaline with sodium hydroxide, the orange colour of the sulphonium salt was discharged, and the purple colour of thionol appeared. Chromatographic analysis revealed the presence of sulphonium chloride, and small amounts of thionol and of phenothiazone. When the urine was hydrolysed by heating with phosphoric acid and was extracted with chloroform, the extract was orange in colour and exhibited the sulphonium chloride spectrum. When the aqueous residue was made alkaline it turned purple; extraction with *n*-butanol showed this pigment to be thionol, giving its strong absorption band at 590–600 mμ.

Discussion

The fate of phenothiazine in the sheep has been investigated in detail. The urinary conjugate has been isolated and identified as potassium leucophenothiazone sulphate, its properties being similar to those of the synthetic compound. Chromatographic and spectrometric analyses have indicated that the pigment in acidified urine is almost entirely phenothiazone. It is therefore concluded that the main excretion product is leucophenothiazone, conjugated with sulphuric acid. Practically quantitative recovery of the drug has been achieved by analysis of the urine and faeces. Oxidation of phenothiazine appears to take place mainly in the rumen, and this organ may be the chief site of absorption also.

In the horse, urinary excretion of phenothiazine is also chiefly in the form of conjugated leucophenothiazone. The fact that less than half of the dosage can be recovered in the excreta has not been explained; this may be due to fixation of the drug in the tissues, or to the formation of derivatives as yet unidentified. The site of oxidation and absorption is unknown, but they may take place in the caecum, which corresponds to the rumen of the sheep in bacterial activity. (In this connection it is of interest to note that the greatest anthelmintic activity of phenothiazine takes place in, and posterior to the caecum; in the sheep this activity commences in the abomasum, immediately posterior to the rumen, and continues throughout the intestinal tract.) An important field for further investigation is the possible relationship between bacterial action, oxidation and absorption, and anthelmintic activity of phenothiazine.

The fate of phenothiazine in the dog appears to be similar to that in sheep and horses, the drug being excreted in the urine chiefly as a conjugate of leucophenothiazone. In the rabbit, thionol was found to be the main excretory product, as reported by DeEds and co-workers (7). In neither of these species were quantitative analyses carried out.

In the pig, as in the rabbit, phenothiazone was found present in acidified urine only in relatively low concentration, thionol being present in much higher concentration. The most striking observation in the pig was the preponderance of free phenothiazine in the acid urine. When concentrated urine is acidified, phenothiazine may precipitate out; if the urine is diluted the phenothiazine apparently dissolves to give the orange coloured sulphonium salt. Very similar results were obtained with humans treated with phenothiazine, the acidified urine containing a large amount of phenothiazine, with smaller amounts of phenothiazone and thionol (6). (In these cases about one-half of a 3 gm. dose was excreted in the urine.)

The fate of phenothiazine in animals appears to be subject to definite species differences. With the data at present available, however, it is impossible to draw any general conclusions as to the relationship between the nature of the excretory products and the type of animal (e.g., herbivorous or carnivorous). One difficulty in interpreting the results of experiments on

urinary excretion lies in the fact that the phenothiazine derivatives are very labile in strongly acid solution, especially in hydrochloric acid, as pointed out by Kehrmann and Diserens (11). These workers found, for example, that the semiquinonoid sulphate of phenothiazine was unstable in free acid, giving rise to a mixture of phenothiazine and phenothiazone. For this reason, the presence of a given derivative in urine treated with hydrochloric acid cannot necessarily be taken as evidence that that compound was excreted in either free or conjugated form. More definite conclusions await the further isolation and analysis of conjugates as they occur in the urine of various species.

References

1. BARNETT, E. DE B. and SMILES, S. J. Chem. Soc. 95 : 1253-1266. 1909.
2. BARNETT, E. DE B. and SMILES, S. J. Chem. Soc. 97 : 186-196. 1910.
3. BERNTHSEN, A. Ann. 230 : 73-211. 1885.
4. BURKHARDT, G. N. and LAPWORTH, A. J. Chem. Soc. 684-690. 1926.
5. COLLIER, H. B. Can. J. Research, D, 18 : 272-278. 1940.
6. COLLIER, H. B. Unpublished observations.
7. DEEDS, F., EDDY, C. W., and THOMAS, J. O. J. Pharmacol. 64 : 250-262. 1938.
8. DEEDS, F. and THOMAS, J. O. J. Parasitol. 27 : 143-151. 1941.
9. GRANICK, S., MICHAELIS, L., and SCHUBERT, M. P. J. Am. Chem. Soc. 62 : 1802-1810. 1940.
10. HILDITCH, T. P. and SMILES, S. J. Chem. Soc. 101 : 2294-2298. 1912.
11. KEHRMANN, F. and DISERENS, L. Ber. 48 : 318-328. 1915.
12. KEHRMANN, F., SPEITEL, J., and GRANDMOUGIN, E. Ber. 47 : 2976-2983. 1914.
13. LIPSON, M. J. Council Sci. Ind. Research, 12 : 342-344. 1939.
14. LIPSON, M. Australian J. Exptl. Biol. Med. Sci. 18 : 269-272. 1940.
15. MICHAELIS, L., GRANICK, S., and SCHUBERT, M. P. J. Am. Chem. Soc. 63 : 351-355. 1941.
16. PUMMERER, R., ECKERT, F., and GASSNER, S. Ber. 47 : 1494-1507. 1914.
17. PUMMERER, R. and GASSNER, S. Ber. 46 : 2310-2327. 1913.
18. SWALES, W. E. Can. J. Research, D, 18 : 266-271. 1940.
19. SWALES, W. E. and COLLIER, H. B. Can. J. Research, D, 18 : 279-287. 1940.
20. SWALES, W. E., COLLIER, H. B., and ALLEN, D. Can. J. Research, D, 20 : 349-361. 1942.

STUDIES ON CESTODES OF THE GENUS *TRIAENOPHORUS* FROM FISH OF LESSER SLAVE LAKE, ALBERTA

I. INTRODUCTION AND THE LIFE OF *TRIAENOPHORUS CRASSUS* FOREL AND *T. NODULOSUS* (PALLAS) IN THE DEFINITIVE HOST, *ESOX LUCIUS*[1]

By Richard B. Miller[2]

Abstract

Triaenophorus crassus Forel and *T. nodulosus* (Pallas) occur in the intestine of the northern pike, *Esox lucius*. *T. crassus* is deeply imbedded in the gut wall of the host; *T. nodulosus* is but lightly attached. Pike weighing three pounds or over are more likely to be infected with the former species and smaller pike more often harbour the latter. The greatest number of mixed infections occur in pike around three pounds in weight.

T. crassus liberates viable eggs into the host's intestine from the end of April to the middle of May; *T. nodulosus* is one month later and appears to liberate its eggs directly into the water. Pike are relatively free of *T. crassus* from mid-May to mid-June and of *T. nodulosus* in late June and early July.

Data presented are interpreted as indicating that the cauda of *T. crassus* is produced by the progressive degeneration of the plerocercoid beginning at the posterior end.

A third, apparently undescribed, species of *Triaenophorus* was found in the intestine of the pickerel, *Stizostedion vitreum*.

Introduction

Cestodes of the genus *Triaenophorus* are the most troublesome tapeworms in Canadian fishes. The plerocercoids of this genus are found in large and conspicuous cysts, frequently in the muscles of valuable food fishes; these cysts often occur in great numbers. Their presence, while not dangerous, is objectionable on aesthetic grounds, and renders the fish containing them practically unmarketable. The Prairie Provinces have suffered greatly as a result of the infestation of fish by these parasites; the once large export of "tullibee" from Lake Winnipeg has been stopped; rigid inspection of Canadian whitefish at the international boundary by United States authorities is now going on, and the refusal of carloads because of this infestation is a common occurrence. Many fine whitefish lakes in Alberta and Saskatchewan have almost ceased to be profitable fishing grounds because of the presence of *Triaenophorus*.

The desire of the Fisheries Branch of the Government of Alberta to arrive at some solution to this problem led to these studies. Lesser Slave Lake was chosen for the work as three species of *Triaenophorus* were found in its fishes; also the provincial whitefish hatchery which provided accommodation and facilities for field work is located on this lake.

During the past two years three species of *Triaenophorus* have been studied. The results of these studies are being presented in a series of papers of which this is the first.

[1] *Manuscript received March 12, 1943.*
Contribution from the Department of Zoology, University of Alberta, Edmonton, Alta.

[2] *Lecturer in Zoology.*

TAXONOMY

Cestodes of the genus *Triaenophorus* have been known as parasites of fresh water fishes since the seventeenth century. Two species are recognized as common in Europe, *Triaenophorus crassus* Forel (1880) and *T. nodulosus* (Pallas) (1760). The genus was first reported on this continent by Cooper (1). His material was collected in Eastern Canada and the United States, much of it from fishes of the great lakes. He referred his specimens to two species, *T. robustus* Olsson (synonymous with *T. crassus*) and *T. nodulosus* (Pallas). *T. robustus* was found in the intestine of *Esox lucius* and *Lota maculosa* and in the muscles of *Leucichthys artedi*. *T. nodulosus* he reported from the intestine and viscera of *Stizostedion vitreum*, intestine of *Esox masquinongy*, from the viscera of *Perca flavescens* and *Micropterus dolomieu*, and from *Catostomus commersonii* and *Notropis delicatus* (organs not specified). Cooper stated that the specimens from the intestine of *Stizostedion vitreum* differed from the others of the *nodulosus* type. In the present study many immature and adult worms from *Stizostedion vitreum* were collected; they have constant differences from *nodulosus* and a different second intermediate host; these worms will be described as a new species.

Hjortland (4) reported *T. robustus* from Minnesota. He found the plerocercoids encysted in the muscle of *Leucichthys tullibee* in several Minnesota lakes; the adult, which he describes in detail, was taken from the intestine of *Esox lucius*.

Wardle (10) described the *Triaenophorus* parasites from Manitoba fishes as belonging to the species *tricuspidatus* Bloch. He distinguished two morphae, *megadentatus* and *microdentatus*. Ekbaum (2) argued that Wardle's material was all *T. crassus;* she believed that his two morphae were based on different views of the scolex hooks of the same species. In the same paper Dr. Ekbaum reviewed the European literature on *Triaenophorus* and showed that the Canadian material that she had studied belonged to the species *crassus*.

Recently the writer sent Dr. Wardle preparations of two species from Lesser Slave Lake; one of these conformed to descriptions of *T. crassus* and the other to *T. nodulosus*. Dr. Wardle reported (personal communication) that these were identical with his two morphae of *tricuspidatus*, the former with morpha *megadentatus*, the latter with morpha *microdentatus*. Thus Dr. Wardle did not, as Dr. Ekbaum surmised, interpret incorrectly two views of the same species, but was actually working with two species. He referred both species to *T. tricuspidatus* Bloch (1779) which is a synonym of *T. nodulosus* (Pallas) (1760). Dr. Wardle believed that two forms with the same definitive host (*Esox lucius*) and differing mainly only in scolex armature, were not sufficiently distinct to be placed in separate species.

While the author has found specimens of *T. crassus* and *T. nodulosus* in the intestine of the same pike on numerous occasions the plerocercoid stages have never been found in the same host or even in the same species of host. Examination of over 500 specimens of *Leucichthys tullibee*, 200 of *Coregonus clupeaformis*, and about 50 *Prosopium* sp. have yielded over a thousand

plerocercoids all of which were intramuscular in position and clearly of Wardle's megadentate form, i.e., *T. crassus*. The plerocercoid of *T. nodulosus* has been found encysted in the liver or other viscera of many varieties of freshwater fishes, but mainly the perch (1). In the present investigation it has been found in the liver of young ling (*Lota maculosa*). Other evidence points to the validity of these two species; data which will be presented later show that there is a distinct correlation between the size of the definitive host (*Esox lucius*) and the species of *Triaenophorus* infesting it. Larger pike tend to be harbourers of *crassus* and smaller pike are more likely to be infected with *nodulosus*.

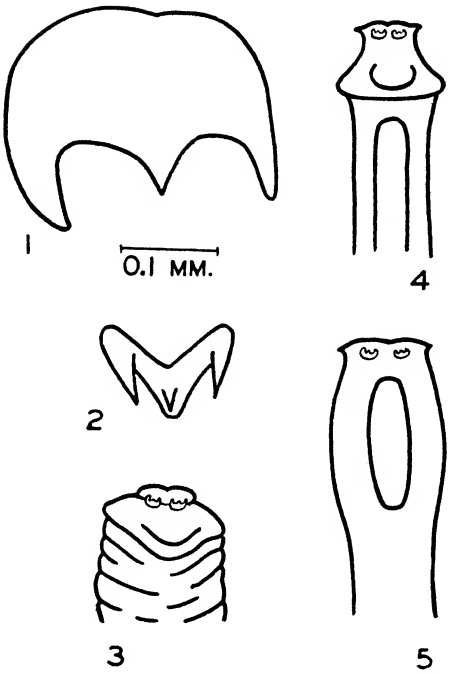
T. nodulosus is the smaller of the two species; in the Lesser Slave Lake material the adults vary from 70 to 270 mm. in length; specimens with developed eggs in the uteri range from 1.5 to 4.0 mm. across the widest part of the strobila. The four hooks on the scolex have a characteristically angled basal plate 112 to 120 μ in width and 20 to 30 μ in depth, the two marginal projections are long and curved; the two median projections are short but both are clearly evident (Fig. 2).

T. crassus is larger in every respect. Mature adults in the Lesser Slave Lake material vary from 130 to 400 mm. in length and from 2.0 to 4.2 mm. in width at the widest point. The basal plates of the scolex hooks are much wider and deeper than in *T. nodulosus*; they measure from 255 to 300 μ wide (nearly three times as wide as in *nodulosus*) and from 132 to 140 μ deep (over five times as deep as *nodulosus*); the marginal projections are relatively shorter, blunter, and much less curved and there is but one clearly recognizable median projection (Fig. 1). The ring-like thickening at the base of the scolex of *T. crassus*, stated by Wardle (10) to be an artifact induced in artificial media, appears, in the Lesser Slave Lake specimens, to be present during moderate or extreme contraction of the scolex, but may vanish during extreme extension (Figs. 3, 4, and 5).

The measurements given above agree closely with those given by Ekbaum (2) for her Canadian material (*T. crassus*) and with measurements of European specimens of both species as quoted by her in the same paper. In view of these facts the writer has no hesitation in following Dr. Ekbaum's lead and referring the material from the pike to the two species, *Triaenophorus crassus* Forel (1880) (= *T. robustus* Olsson, 1893, = *T. tricuspidatus* morpha *megadentatus* Wardle, 1932) and *Triaenophorus nodulosus* (Pallas) (1760) (= *T. tricuspidatus* Bloch, 1779, = *T. tricuspidatus* morpha *microdentatus* Wardle, 1932).

Biology

The biology of triaenophorine tapeworms has been quite thoroughly studied in Europe. Scheuring (9), who brought together the observations of many authors and the results of his own extensive investigations, has given a fairly complete picture of the biology of *T. crassus* and *T. nodulosus* in European lakes and fishes. Much less has been accomplished on this continent. Nicholson (8) reported the presence of the plerocercoids of a *Triaenophorus* parasite



NOTE: *The line between Figs. 1 and 2 represents 0.1 mm. and is the scale to which these two figures are drawn; Figs. 3, 4, and 5, not drawn to scale.*

FIG. 1. *Camera lucida drawing of a scolex hook of Triaenophorus crassus.*

FIG. 2. *Camera lucida drawing of a scolex hook of Triaenophorus nodulosus.*

FIG. 3. *Sketch of the contracted scolex of Triaenophorus crassus.*

FIG. 4. *Sketch of the moderately extended scolex of Triaenophorus crassus.*

FIG. 5. *Sketch of the fully extended scolex of Triaenophorus crassus.*

in the flesh of the tullibee; he proved that these were non-pathogenic to dogs. In the same paper he discussed the similarity of the scolices of worms found in the pike intestine to those of the plerocercoids and concluded that they were the same. Newton (7) examined coregonine fishes from numerous Manitoba lakes; he studied seasonal changes in the number of plerocercoids in the flesh. He also examined "*Triaenophorus tricuspidatus*" from the gut of *Esox lucius* throughout the year and made observations on times of maturity. He was unable to discover the first intermediate host. Ekbaum (3) studied *Triaenophorus crassus* in the gut of the pike throughout the year; her observations on time of maturity fail to confirm Newton's. She also observed the hatching of the eggs and the dimensions of the coracidia.

These studies leave the identity of the first intermediate host of *T. crassus* unknown and contain contradictory evidence on other points of the life history. Nothing on the biology of *T. nodulosus* has yet been reported by investigators on this continent. The immediate object of the present investigation has

been, accordingly, the amplification of and addition to the knowledge of the biology of the genus.

By way of introduction to what follows in this and other papers a brief outline of the life history of the three species found in Lesser Slave Lake is presented.

T. crassus and *T. nodulosus* occur as adults in the intestine of the pike, *Esox lucius*. In the spring the eggs are released and the parasites leave the host and die. The eggs hatch into ciliated, free-swimming coracidia of microscopic size; these infect Copepoda of the genus *Cyclops*. The next host of *T. crassus* is a coregonine fish which becomes parasitized by eating an infected *Cyclops*. The genera *Coregonus*, *Leucichthys*, and *Prosopium* are the coregonids of Canadian waters and all are suitable hosts. In these fishes the parasite is a plerocercoid encysted in the muscles and spoiling the flesh for market purposes. The life history is completed when a pike swallows a fish containing plerocercoids.

The second intermediate host of *T. nodulosus* is usually the perch in which the plerocercoid is found encysted in the liver (5). In Lesser Slave Lake the plerocercoids of this species were found in the liver of young ling (*Lota maculosa*). The completion of the life cycle is achieved when a pike swallows an infected ling. *T. nodulosus* lacks, therefore, the economic significance of *T. crassus* since its plerocercoids, being intrahepatic, do not influence the marketability of the fish.

The third species of *Triaenophorus*, which is apparently undescribed, occurs as an adult in the pickerel (*Stizostedion vitreum*). The first intermediate host is again a *Cyclops*, and the second, the trout perch (*Percopsis omiscomaycus*), in which the plerocercoid encysts in the liver.

The Life of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas) in the Definitive Host, the Northern Pike (*Esox lucius*)

Location and Appearance in the Host

Triaenophorus crassus and *T. nodulosus* are both found in the intestine of the host. The scolices are attached to the intestinal wall not far below the pylorus; the strobilae extend into the lumen distad from this point. In recent infections the strobilae are inconspicuous and are often hidden in the copious intestinal mucus; later they become very prominent and, when maximum development has been reached, may fill the lumen completely. The strobilae of *T. nodulosus*, especially those of ripe specimens, are commonly coiled together in a tight mass in the centre of the lumen. This association may be so intricate that it is difficult to untangle and secure a whole, undamaged specimen. The strobilae of *T. crassus* typically lie much more loosely associated; single specimens are often found, however, that have tied themselves into quite complex knots. As full maturity approaches, the strobilae of both species become straighter and eventually stretch the full length of the intestine, sometimes protruding into the rectum.

The scolex of *T. nodulosus* is but lightly attached to the gut wall; the hooks are imbedded at the bottom of one of the folds of the mucosa; the mucosa is often destroyed right at the point of attachment (Fig. 6). One can take hold of a mass of these worms and lift them easily from the intestine; no scar or wound can be detected (with the unaided eye) where they were attached. Recent infections of *T. crassus* affect the host just as little; well established infections of this species have a marked effect. Each scolex is buried deep in the intestinal wall; in some cases the whole of the mucosa and submucosa is penetrated and the scolex lies against the circular muscle layer. Two photomicrographs of sections through a typical *crassus* attachment site are shown in Figs. 7 and 8. At the point of attachment of each worm the mucosa is missing and the submucosa is greatly thickened; when the scolex is withdrawn this thickening presents the appearance of a small hillock with a hole in its centre. These hillocks with their pits persist for some time after the parasites have left and are very useful evidence, in the spring, that a pike has been infected with *T. crassus* and has subsequently lost them.

In both species the bothria appear to be of very little use in anchoring the cestodes to the gut wall; sections through these structures (with the worm imbedded in the gut wall) show that the surrounding tissue is not drawn into them.

Mixed Infections

While mixed infections of *T. crassus* and *T. nodulosus* are common in Lesser Slave Lake, there is a distinct tendency for the larger pike to have mostly or only *T. crassus* and the smaller hosts to have a larger proportion of *T. nodulosus*. The incidence of both species in 100 infected pike of all sizes is shown in Table I.

TABLE I

THE INFLUENCE OF THE SIZE OF THE HOST (*Esox lucius*) ON ITS POPULATION OF *Triaenophorus* PARASITES

Weight of host (pounds)	Up to 1	1.1 to 1.5	1.6 to 2.0	2.1 to 2.5	2.6 to 3.0	3.1 to 3.5	3.6 to 4.0	Over 4
With *T. crassus*, %	20	20	23.5	26.7	45	100	88.9	93.3
With *T. nodulosus*, %	80	90	94.1	86.7	85	25	22.2	26.7
With both species, %	0	10	17.6	13.4	30	25	11.1	20.0
Average no. *T. crassus* per host	1	2.5	1.5	6	3	13	18	21
Average no. *T. nodulosus* per host	4.5	6	5	7	6	3	1.5	4

Less than half of the infected pike of three pounds and under contained *T. crassus*; the smallest pike were the least infected with this species. Close to all pike over three pounds in weight harboured *T. crassus*; the heavier the host, the greater the number of individuals it contained. In *T. nodulosus*

the reverse is true; 80 to 95% of infected pike three pounds or under, and only about 25% of those over three pounds, contained this species. The number of individuals of *T. nodulosus* tends to be about twice as great in pike three pounds and under as it is in heavier fish.

Mixed infections occur in about 30% of the infected pike weighing from two and a half to three pounds. Fewer mixed infections occur in hosts either larger or smaller than these. Scheuring (9) remarks on a somewhat similar distribution according to size in Europe; he found that pike four pounds and over seldom harboured *T. nodulosus*, but contained large numbers of *T. crassus*.

This tendency for the two species to occur in different sized hosts is a natural outcome of the distribution of the plerocercoids; *T. nodulosus*, which encysts in the liver of young ling, small fish, is swallowed more often by small pike; *T. crassus*, which encysts in whitefish and tullibee, larger fish, is swallowed more often by larger pike.

The above noted facts lend weight to the belief that *T. crassus* and *T. nodulosus* are distinct species, and tend to invalidate Dr. Wardle's contention that their similar host distribution is an indication that they are but morphae of one species.

Seasonal Changes

Over a 12 month period an average of 28% of all Lesser Slave Lake pike are infected with *T. crassus* and 47% with *T. nodulosus*. The greatest percentage infection occurs in the spring and the least in early summer (Table II). Michajlow (6) found 76% of the pike in the Warsaw fish markets were infected with *T. nodulosus*.

The numbers of both species found in each infected pike vary considerably. The average for *T. crassus* is 12 per host with a range of from 1 to 70. *T. nodulosus* averages five per host with a range of from 1 to 94.

The size of the parasites in the host's gut varies for both species throughout the year. The largest specimens of both are found in March, April, and early May in Lesser Slave Lake. The smallest individuals are found in late May and early June.

In Table II the sexual development of both species at different times of the year is shown. The anlagen of the genitalia of *T. crassus* probably appear in November; the first eggs must appear in the uteri in January; by February the uteri are distended with eggs but these do not show the outlines of the embryos. During March and April the onchospheres become visible in the eggs. The worms first begin liberating eggs when placed in water in April. On April 22, 1942, one out of 21 specimens examined that day released eggs in which the embryos could be seen moving; these eggs hatched on April 24. This is the earliest date recorded for Lesser Slave Lake specimens. The majority lay their eggs during the first half of May; pike examined every two or three days from May 1 to 15 contained *T. crassus* in the various stages of egg laying. In the early phase the worms are at their maximum size and extend, without kinks, full length from their points of attachment. Such

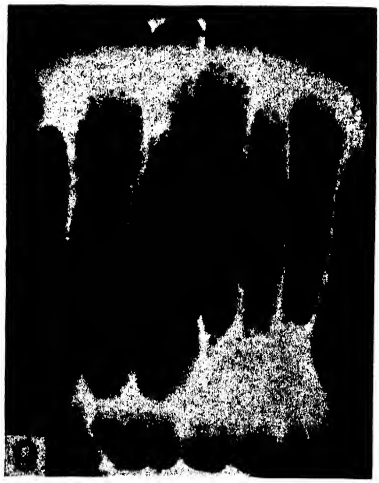


FIG. 6. *Photomicrograph of a section of the intestine of Esox lucius through the site of attachment of an adult Triaenophorus nodulosus. One of the scolex hooks of the worm is visible.* ×27. FIG. 7. *Photomicrograph of a section of the intestine of Esox lucius through the point where an adult Triaenophorus crassus has penetrated.* ×16. FIG. 8. *Photomicrograph of the same specimen shown in Fig. 7; in this section the scolex of the parasite is visible; one of the hooks can be distinguished.* ×21. FIG. 9. *Photomicrograph of a portion of the strobila of a fully ripe Triaenophorus crassus. The large uteri, full of eggs, are very conspicuous.* ×17. FIG. 10. *Photomicrograph of a portion of the strobila of a partially spent Triaenophorus crassus. The empty and partly empty uteri can be seen.* ×26.

TABLE II

SEASONAL VARIATION IN NUMBERS AND THE STATE OF THE SEX ORGANS OF *Triaenophorus crassus* AND *T. nodulosus*

Date 1941 and 1942	No. pike examined	With *crassus*, %	With *nodulosus*, %	Sexual development	
				T. crassus	*T. nodulosus*
February	24	70	70	Eggs in uteri	Eggs beginning to appear
March	12	50	50	Eggs in uteri, embryos outlined	Eggs in uteri
April	24	29	67	Embryos outlined in eggs; eggs released in water; some hatching	Eggs in uteri; embryos not yet outlined
May 1–14	40	37.5	57	Releasing eggs or spent and dying	Beginning to release eggs; embryos outlined in some
May 15–31	57	8.8	5.3	Spent and dying and fresh infection	Liberating eggs; some hatching
June	64	15.6	25	A few spent and dying; fresh infection	Liberating eggs; dying
July	2	50	0	Immature	
September	12	33.3	16.7	No trace of genitalia	No trace of genitalia
October	4	100	25	No trace of genitalia	No trace of genitalia
December	8	25	87.5	Genitalia well formed, no eggs in uteri	Anlage of genitalia

worms, placed in water, release clouds of white eggs* which hatch in two or three days. A few days later the appearance of the worms has changed; their strobilae are still straight but are very irregular in diameter, particularly at the posterior end which may be reduced from 4 mm. to 1 mm. in breadth. Such worms are actively releasing eggs and the resulting empty uteri cause the collapse of the strobilae (Figs. 9 and 10). During this period many eggs may be found in the faeces of the host; these eggs hatch in a few hours when placed in water. By May 15 few *T. crassus* can be found in the pike intestine; those present are fragmented and spent; often only the scolex, still imbedded, and about a half inch of the strobila are found. Many pike examined during this period had empty pits in the intestinal wall which indicated that the parasites had been there a short time previously. Only five pike containing *T. crassus* were found in 57 examined during the latter half of May (1941 and 1942). Of these, three contained only the scolices and short bits of the strobilae; the other two were clearly infected with the new generation—small, entire worms, very lightly attached to the gut wall.

* *Ekbaum (3) stated that the ripe eggs of T. crassus are brown and that the uteri of mature specimens show up as a row of brown dots along the strobila. The author has never found brown eggs in the uteri of a worm still in the host's gut. Within half to three-quarters of an hour after placing the worms in water, however, the eggs turned brown and those still in the uteri gave the appearance noted by Dr. Ekbaum.*

During the first two weeks in June the pike are relatively free of *T. crassus*, but by the middle of the month several were found with fresh infections of small worms. There is, therefore, a period of about a month from mid-May to mid-June when the pike are almost free of *T. crassus*.

The anlagen of the genitalia of *T. nodulosus* are visible by December; eggs appear in the uteri in February; eggs containing formed onchospheres are first liberated in May. The earliest hatchings were recorded on May 31, 1941, from eggs collected from uteri on May 26. This species is thus about one month later than *T. crassus*. The main egg laying period for *T. nodulosus* is toward the end of May and in early June.

On May 31, 1941, a pike was found with one *T. nodulosus*; the worm was protruding from its anus; on June 1 two of three pike examined had this species; one had the parasite protruding from its anus, the other had the worm lying, unattached, in the rectum. On June 2 another pike was found with a ripe specimen of *T. nodulosus* partially out its anus. In all these fish the worms were entire and had not begun laying eggs; in other fish only portions of the strobilae were found in the rectum, but the uteri of these portions were full of eggs. A worm or portion of one, taken from the rectum or anus of the host and placed in water, began immediately to discharge clouds of eggs. It thus seems evident that *T. nodulosus* differs somewhat from *T. crassus* in its method of releasing its eggs; either the entire worms or fragments of them pass out the host's anus into the water *before* releasing their eggs. The eggs are released in the water and the spent worms or fragments sink to the bottom. Such spent worms, still entire, were found on the bottom of a tank where pike had been kept under observation during this period.

During the last three weeks of June only two specimens of *T. nodulosus* were found in 20 pike examined during this period in 1941 and three in 17 pike examined during the same period in 1942. All three in 1942 appeared to be new infection.

Michajlow (6) examined pike from Warsaw fish markets and found adults of *T. nodulosus* in the winter and early spring and immature worms at all other times. These findings are in agreement with the data reported here for this species.

The absence of *T. crassus* and *T. nodulosus* from the pike gut for a short period following egg laying has been observed in Europe (9); the time appears to be correlated with latitude; maturation and egg laying occur earlier in more southern localities.

These data on the time of maturation of *T. crassus* largely confirm similar data secured by Ekbaum (3) from Lake Winnipeg, Lake Nipissing, and Lake of the Woods, but fail to agree with Newton (7) who found no sexual development from May to August and mature eggs in the uteri in February.

The Cauda of *Triaenophorus crassus*

At any time during the year when *T. crassus* is present in the pike gut immature specimens may be found. In May, specimens that show no trace

of genitalia coexist with mature and spawning individuals. These immature worms appear to be lost with the others when spawning is over. As pike feed during the whole year the presence of these young worms at all times is not surprising. The most recently ingested are often provided with a cauda; this is a posterior appendage of variable length but always of a lesser diameter than the rest of the strobila. Histological preparations reveal that the muscle and parenchyma of the cauda are degenerate. The cauda varies considerably in size in different individuals. In May and July Scheuring (9) found caudae from 0.5 to 20 mm. in length; Cooper (1) found caudae of variable length in *T. crassus* from the pike; one of his specimens, 48 mm. long, possessed a cauda of 24 mm. The cauda is apparently shed if large, or absorbed if small, soon after the worm is established in the definitive host. This structure is frequently found as an appendage of the encysted plerocercoid; there is evidence that its size is dependent on the age of the plerocercoid. Recently encysted plerocercoids have either no cauda, or a very small one; plerocercoids that have been encysted for several months may have caudae over half their total lengths. The seasonal variation of this appendage in specimens from the pike gut tends to bear this out. Thus of 23 recently ingested specimens of *T. crassus*, collected May 29 and having an average total length of 43 mm., only three possessed caudae. Eight worms, 40 to 75 mm. long, obtained June 19, had no trace of caudae, although they were clearly very recent arrivals in the host's intestine. Both these lots were obtained at a time of year when the plerocercoids are very recently formed in the flesh of the second intermediate host.

Twenty-six recently established *T. crassus* larvae were removed from pike in September. These worms averaged 57 mm. long; 13 possessed caudae. The encysted plerocercoids from which these larvae came would be three or four months old.

In February, 26 specimens of *T. crassus* that appeared to be recently ingested were secured. These averaged 95 mm. in length; 16 of them possessed caudae. These worms developed from plerocercoids that were eight or more months old.

In April and early May, 14 *T. crassus* larvae, recently established, were found in pike. These averaged 97 mm. in length; caudae were present on 12 of them. These worms developed from plerocercoids that were 10 or more months old.

The length of the cauda in specimens taken from the pike gut cannot be used; the cauda of nearly every worm shows a frayed end, indicating it is disintegrating; the original length, therefore, cannot be obtained. In general the longest caudae were found in the spring. Thus in February several of 75 mm. were measured and these were incomplete.

It would seem that the older the plerocercoid the greater is the probability of a cauda being present, and the longer it is likely to be. Some encysted plerocercoids have been found in the spring which were dead in their cysts and on which the cauda made up almost the entire strobila; in still others only the disintegrating scolex was found. The author believes that the cauda is not

a true appendage but that it is produced by the gradual disintegration of the strobila, starting at the posterior end; examination of living specimens reveals that the worms are incapable of moving their caudae; irrespective of the state of contraction of any individual, its cauda remains the same length. Specimens whose caudae have reached more than half the total length of the strobila are usually dead or dying.

The author has not observed an appendage of this kind in *T. nodulosus*.

Acknowledgments

The writer is happy to acknowledge the generosity of Dr. Ella Ekbaum, of the University of Toronto, who loaned him copies of the text and figures of papers by Scheuring and other European authors which she had made for her own use. He is also grateful to the Department of Lands and Mines of the Government of the Province of Alberta whose financial aid has made this work possible and is especially indebted to Messrs. H. B. Watkins and H. V. Dempsey of the Fisheries Branch of the same Department who arranged for the delivery of fish and the use of much of their gear.

References

1. Cooper, A. R. Illinois Biol. Monogr. 4 : 289-541. 1918.
2. Ekbaum, E. J. Parasitol. 21 : 260-263. 1935.
3. Ekbaum, E. J. Parasitol. 23 : 293-295. 1937.
4. Hjortland, A. L. J. Parasitol. 15 : 38-44. 1928.
5. Janicki, C. Korresp.-Bl. Schweiz. Aerzte, 48 : 1343-1349. 1918.
6. Michajlow, W. Ann. parasitol. humaine comp. 11 : 339-358. 1933.
7. Newton, M. V. B. Contrib. Can. Biol. Fisheries, 7(28) : 341-360. 1932.
8. Nicholson, D. Can. J. Research, 6 : 162-165. 1932.
9. Scheuring, L. Z. Parasitenk. 2 : 157-177. 1929.
10. Wardle, R. A. Contrib. Can. Biol. Fisheries, 7(30) : 377-403. 1932.

NOTES ON THE LIFE HISTORY AND MORPHOLOGY OF *CEPHENEMYIA JELLISONI* TOWNSEND AND *LIPOPTENA DEPRESSA* SAY, TWO DIPTEROUS PARASITES OF THE COLUMBIAN BLACK-TAILED DEER (*ODOCOILEUS HEMIONUS COLUMBIANUS* (RICHARDSON))[1]

By Ian McT. Cowan[2]

Abstract

Material derived from deer taken on southern Vancouver Island, B.C., represents all larval stages of the nostril fly, *Cephenemyia jellisoni* Townsend. Description is given of the external morphology of the three larval stages and the puparium of this fly. The tracheary system of the first instar, and the cephalopharyngeal apparatus of all three are described and figured.

In November and December first instar larvae were found in the nasopharynx of the host where they remained until after the moult. Until they reach maturity, second and third instar larvae occupy the retropharyngeal recesses of the deer. They leave the host by way of the nostrils and pupate in the ground.

Observations on the life history and behaviour of *Lipoptena depressa* Say, both on and off the host, are given. It is postulated that the life span on the host varies from 8 to 13 months and that during this period from four to seven larvae are produced. Larvae do not pupate on the host but fall to the ground as soon as they are liberated. Infestations on a single host may consist of more than 2000 flies; under such circumstances the deer evinces discomfort. As yet this fly is not known to be involved in the life cycle of any internal parasite of the deer.

During several years devoted in part to a study of the life history and ecology of the Columbian black-tailed deer (*Odocoileus hemionus columbianus* (Richardson)) a considerable body of data has been assembled concerning the life histories of certain parasites of this mammal. Inasmuch as some of these data appear to supplement the rather meagre existing information on the two dipterous parasites of this deer but are at the same time unsuitable for inclusion in a larger paper, in preparation, on the parasites and diseases of this host, they have been compiled into this separate report.

The Deer Nostril Fly, *Cephenemyia jellisoni* Townsend

This fly is an important parasite of the deer that inhabit the coastal regions of British Columbia where it has been enountered by the writer as far north as Porcher Island. The same or a closely related species parasitizes the mule deer (*O. hemionus hemionus*) of central British Columbia.

The specific identification of the bot fly larvae recovered during this study rests on unsatisfactory grounds. Though adult flies were observed on the wing, none was captured. A single adult fly from Vancouver Island, in the University of British Columbia collection, has been provisionally identified

[1] *Manuscript received January 11, 1943.*
Contribution from the Department of Zoology, The University of British Columbia, Vancouver, B.C.
[2] *Professor of Zoology.*

as *Cephenemyia jellisoni* by Dr. D. G. Hall of the U.S. National Museum. Then, too, on the basis of key characters set forth by Townsend (7), third instar larvae fall closest to *C. jellisoni* in that the dorsal spines are larger than the ventral, and the mouth hooks are strongly recurved. All the larvae reported upon here, unless otherwise stated, were collected from deer taken on southern Vancouver Island, B.C., in the vicinity of Victoria and Duncan.

The intimate details of oviposition have not been observed but by analogy with other species of the genus it can be assumed that minute living larvae are deposited on the nose or within the nostrils of the deer. The activities of the female flies observed during oviposition induce violent reactions on the part of the host. Deer under attack toss their heads, sometimes jump up and down stiff-legged in one place, and have been seen to screen their nostrils by placing them against the ground and beneath the back of the front foot.

On southern Vancouver Island adult flies have been observed on the wing during July and August, but indications are that the flying season is longer than that and probably extends from early June to late September.

Larvae of the first instar were recovered from the nasopharynx and nasal chambers of the host during November and December. Here they were found actively moving over the mucous membranes covering the ethmoturbinates and the nasal surface of the soft palate. None was found in the frontal or maxillary sinuses.

Apparently the first ecdysis takes place in the nasal cavity and it is the early second instar larva that migrates to the retropharyngeal recesses of the host. A deer taken February 22, 1942, contained 17 larvae, 13 second instar and four third instar. The extended length of the former ranged from 5.5 mm. to 14.2 mm. and of these the smallest was still in the nasopharynx. The rest were in the retropharyngeal recesses, the invariable site of all other second instar larvae obtained. The earliest collections of second instar larvae were made in a deer taken at Goldstream Lake, Vancouver Island, B.C., on February 22, 1940, but inasmuch as in this deer only three of 19 larvae were in the second instar, while 16 were already in the third instar and the largest 28 mm. in length, it is obvious that in many individuals the first moult must be undertaken in early January, possibly as early as December. May 21 was the last date upon which any second instar larvae were present, but on this date the smallest was still just 7 mm. in length.

The second ecdysis leading to the third instar larva takes place at a length of 14 to 15 mm. Larvae of this stage grow to an extreme length of 35 mm. and during this period occupy the retropharyngeal recesses of the host as do those of the second instar. There are records in the literature of third stage larvae elsewhere in the head and respiratory passages of the host but the writer's experience has been that these abnormally situated larvae usually are the result of wanderings after death of the host. Under such circumstances larvae have been removed from the lungs, trachea, oesophagus, nasal passages, and mouth, whereas in all deer specimens examined immediately after death the larvae have been confined to the pouches.

The presence of 28 mm. larvae in a deer taken on February 22 indicates that the third instar is reached, by some at least, as early as January. In a collection made on June 13, there were no larvae less than 24 mm. in length, 8 of 15 were over 30 mm. in length and heavily pigmented. Deer examined in mid-July were not parasitized by this fly.

As the larvae approach maturity the pigmentation of first the spinose areas, later the entire integument, becomes greatly augmented so that instead of yellowish white, studded with brown spines, the mature larva becomes brown set with blackish spines.

Passage from the host was not observed under normal circumstances but those leaving the host after death did so by way of the nostrils. Doubtless, as stated by Hadwen (3) for *C. trompe*, this passage from the host is assisted by the unfortunate animal's sneezing. The earliest mature larvae recovered were taken on March 19, 1940, at Chemainus, Vancouver Island, B.C.

The writer has been successful in getting only two larvae to pupate. These burrowed to a depth of 3 to 4 in. in moist earth. Pupation took 48 hr. and pigmentation was not complete until 72 hr. had elapsed. Attempts to rear adult flies from these puparia failed but Jellison (4) succeeded under similar circumstances with larvae obtained from mule deer. Incubation periods of pupae were 56 and 61 days.

Notes on the External Morphology of Larval Stages of *C. jellisoni*

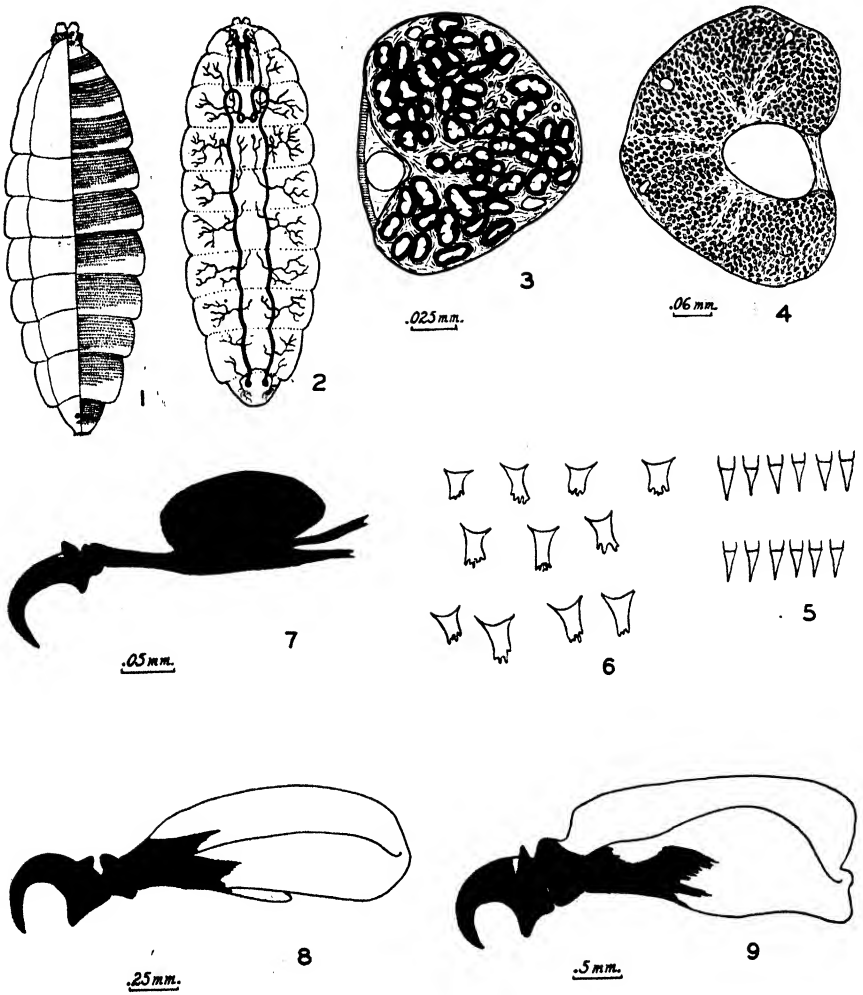
First Instar Larva

The first instar larvae obtained during this investigation range from 1.5 to 3.5 mm. in length, the greatest breadth occurring at the 7th to 10th segments. Cephalic end narrows gradually, caudal end abruptly rounded. Ventral surface flattened, dorsal surface strongly arched on mid-line (in transverse section) and with flattened marginal lappet on each lateral border. Ventral surface provided with a series of parallel transverse rows of short triangular spines arranged on the anterior part of each body segment from IV to XIII. Dorsal surface spineless, except for first postoral segment, integument smooth (Fig. 1).

Cephalic segment slightly broader than long and provided with a pair of short, bluntly rounded antenniform processes projecting anteroventrally parallel to each other; apices bearing two minute slightly sclerotized papillae. Buccal hooks black, strongly recurved.

Segment II not quite twice as broad as long, provided with a ruff-like tuft of spines directed posteriorly and extending along anterior margin from mid-dorsal line to base of buccal hooks on each side. These tufts broadest laterally (11 rows) and tapering toward their ventral termination; anterior spines longest, posterior shortest. Another patch of short spines mid-ventrally located in a roughly circular area toward posterior boundary of segment. Segment III bare.

Segments IV to XII become progressively longer. Segment XIII bluntly rounded and provided terminally on its ventral surface with single row of black, strongly curved spines, 10 to 14 in number; the external spines shortest, the median ones longest.



FIGS. 1 – 9. *Cephenemyia jellisoni.* FIG. 1. *First instar larva of Cephenemyia jellisoni, left half as seen from dorsal aspect, right as seen from ventral aspect.* FIG. 2. *Dorsal view of tracheary apparatus of first instar larva.* FIG. 3. *Surface detail of right posterior spiracle of second instar larva.* FIG. 4. *Surface detail of left posterior spiracle of third instar larva.* FIG. 5. *Details of ventral cuticular spines on first instar larva.* FIG. 6. *Details of ventral cuticular spines on second instar larva.* FIG. 7. *Cephalopharyngeal apparatus of first instar larva.* FIG. 8. *Cephalopharyngeal apparatus of second instar larva.* FIG. 9. *Cephalopharyngeal apparatus of third instar larva.*

All segments except I, II, and III armed ventrally with parallel rows of short, scale-like, triangular (when viewed ventrally) spines that are not differentially pigmented (Fig. 5). Each is supported basally on a small rectangular scale imbedded in the integument. The number of these rows varies in the specimens on which they were counted. In six specimens the number was as follows: Segment IV—6; V—9; VI—10 to 11; VII—12; VIII—12 to 13; IX—12 to 14; X—13 to 14; XI—12 to 14; XII—12 to 14; XIII—12. In two other specimens the segments had respectively 6, 7, 6, 6, 6, 7, 8, 8, 8, and 9 spine rows. Inasmuch as others from the same locality had the greater number of rows it is possible that these two specimens represent another species of *oestrid* despite the similarity in structure of their mouth hooks and other anatomical features.

In view of the speculations voiced by Cameron (1) and others as to the probable function of the terminal proleg it is significant to note that the terminal hooks on Segment XIII of larvae examined by the writer are curved anteriorly so as to fit them for resisting forward movement rather than to assist progress in this direction.

Cephalopharyngeal Apparatus

Buccal hooks long, slender, smooth, ventrally recurved, and divergent at their pointed distal extremities. According to Cameron (1), hooks in this genus are composite, the expanded base being composed of fused dorsal hypopharyngeal and ventral anterior transverse sclerites (Fig. 7).

Viewed from the ventral aspect the hypostomal sclerite is an open *U* shape with the anteriorly directed arms of the *U* broadened dorsoventrally to articulate with the base of the buccal hooks. Posteroventrally the two arms unite with each other and the basilar sclerite. Directed anteriorly from each side of the union, medial to the lateral arms, are two short, conical projections, and anterior and ventral to these is an imperfectly sclerotized structure the significance of which it has not been possible to determine.

Basal sclerite heavily sclerotized ventrally; anteriorly a long slender process meets the hypostomal sclerite; broadened middle part deeply incised posteriorly into dorsal and ventral posteriorly directed slender cornua.

The writer has been unable to detect the presence of a lateral accessory process though in one specimen there is a short lateral protuberance of the basal sclerite that may possibly be such in a rudimentary condition.

Tracheary Apparatus

The first instar larva is metapneustic. The posterior stigmata are situated on Segment XIII, dorsal to the anal opening. The dorsal respiratory trunks proceed anteriorly, giving rise to a lateral branch into each body segment from XIII to V. In Segments V and VI they are folded back upon themselves, pass ventrally and then anteriorly to terminate in an arborescence in the region of the posterior end of the pharynx. Two parallel trunks pass anteriorly from the anterior-most loop of the dorsal trunk on each side, the

internal ventral to the external. Eight lateral trunks arise from each dorsal trunk. These all branch ventrally, medially, and laterally, the lateral branch arborescing. Just anterior to the origin of the terminal pair of laterals a fine connective passes directly across from one dorsal trunk to the other. Another commissure joins the two dorsal trunks in Segment VI (Fig. 2).

From the ventral aspect can be seen a series of nine fine paired canals each arising dorsally and passing more or less directly to the ventral surface of the larva near the mid-line. Here each passes posteriorly then laterally where each trunk is united with those anterior and posterior to it by irregular, delicate connectives that in all probability represent the degenerate lateral tracheal trunks.

Second Instar Larva

The writer's collections include only few very young second instar larvae but all specimens whose total length is from 5.5 mm. to 14.5 mm. are in this stage. Second instar larvae are elongate, semicylindrical, increasing in transverse diameter gradually until the 9th or 10th segment and then decreasing slightly to the 13th or caudal segment. The ventral surface is somewhat flattened and the dorsal surface, arched. The body is broader than high except in Segment XIII. This ultimate segment is equipped ventrally with a strongly developed proleg and bears dorsally a transverse crescentic elevation. The posterior face of this elevation extends ventrally and slightly anteriorly as an almost flat surface to meet the proleg at an angle of about 140 degrees. Posterior spiracles are situated just dorsal to this angle. Colour white until the moult is near, then becoming yellow.

Exoskeleton

The anterior segment (I) bears a pair of anteriorly directed antenniform processes which take the form of short bulbous projections with bases approximated and tips divergent. As in the first instar there is a pair of small chitinous plates on the summit of each antenna and as an addition a third brown chitinous plate on the ventral surface at the base of each antenna.

Segment I unarmed, separated from II by a deep sulcus armed on its posterior face with several rows of brown, posteriorly directed spines, arranged in three groups. A dorsal patch of five rows of long spines (those of anterior row longest, of posterior row shortest) extends corona-like from side to side across the dorsum, above the antenniform processes, and a small triangular ventrolateral group on each side extends lateral and slightly anterior to the mouth hooks. These groups are composed of nine rows ($\pm$) of small strongly hooked spines. Segments II and III (first and second postorbital), indistinguishably united, bear upon their dorsal surfaces a subquadrangular naked area, the cervix, bounded anteriorly by a transverse furrow and laterally by a single boss-like elevation on each side that extends over the lateral surface ventrally and then posteriorly to terminate opposite the mouth hooks. A minute anterior spiracle is situated just dorsal to midlateral line on posterior margin of this segment. There are about two dozen micro spots on the cervix.

Segments IV to XI bear on the anterior part of their ventral surfaces parallel, transverse rows of short, scale-like, posteriorly directed spines, each with its distal extremity castellate and flat rather than pointed (Fig. 6). These rows number 6 on Segment IV; 8 on V; 9 on VI; 12 on VII; 11 on VIII; 12 on IX; 12 on X; 11 on XI. Segments XII and XIII are naked ventrally; proleg on XIII is provided with 5 or 6 rows of small proclinate spines, 5 rows of very small spines at the posterior base of the proleg.

Unlike the first instar larvae those of the second stage have the dorsal and lateral surfaces armed with spines; this is doubtless associated with the change from the free wandering of the first instar larva over the mucous membranes of the nasopharynx to the existence in the circumscribed crypts of the retropharyngeal recesses undertaken by the second instar larva. The large posteriorly directed dorsal and lateral spines of this stage are an important contribution to the maintenance of position whereas they would be useless to the first instar larva in which only the ventral surface contacts the host.

The stout, sharp-pointed dorsal spines are in roughly parallel, but sinuate, rows on the anterior part of each segment, except XIII, and are reclinate except on Segment XII. The number of these rows is as follows: Segment IV—3 to 4; V—3 to 5; VI—3 to 5; VII—3 to 5; VIII—3 to 5; IX—3; X—3 to 5; XI—2; XII—3 to 5 at the posterior edge, proclinate; XIII—naked. Spines of anterior larger than those of posterior rows. On each side of the anterior part of Segments IV to VI are small, almost circular spinous areas separated from the dorsal spinous area by a narrow naked patch. This naked patch is less pronounced but present on the remaining dorsally-armed segments and on these the lateral areas are correspondingly less distinct. Each segment from V to XI bears on each side along its posterior edge a single row of spines originating opposite the lateral areas and extending to varying degrees onto the dorsum.

Posterior stigmata dark brown, roughly triangular, slightly convergent ventrally and studded with elongate, irregularly shaped pores (Fig. 3).

Cephalopharyngeal Apparatus

Mouth hooks large, more strongly everted at the tips than in either the first or third instar, anterior transverse portion very large, much larger than the hypopharyngeal portion, bases of spines slightly rugose externally from the dorsal aspect. A small dentate sclerite is loosely associated with the medial surface of each hook.

Hypostomal sclerite similar to that of the first instar except that the ventral union is more extensive and anterior projections are wanting. Basal sclerites are heavily sclerotized anteriorly and broadly joined ventrally at the anterior extremity. They differ markedly from the first instar in absence of posterior incision, restriction of sclerotization to anterior part and presence of a well developed lateral accessory process (Fig. 8).

Third Instar Larva

The larva reaches a length of about 35 mm., increases in transverse diameter until the sixth segment, the width being maintained to the 10th segment, then tapers gradually to the caudal extremity. Ventral surface flattened and the dorsal, vaulted.

There are 14 segments; I (pseudocephalon) minute, without spines or denticles and bearing two short antenniform processes; separated from Segment II by a deep sulcus that bears on its posterior face a ruff-like band of reclinate spines, the ventral ones minute, in four to five rows, increasing in size toward the dorsum where about eight large spines constitute the anterior row. Posterior part of Segment II bare on dorsal surface but with a median longitudinal suture. Segment III without spines; cervical area on its dorsal surface slightly broader than long and with from 24 to 36 dark brown, circular plates imbedded in the cuticle in three approximately longitudinal rows. Segments II and III externally indistinguishable ventrally. Three boss-like eminences in a vertical row on each side of cervical area, derived by subdivision of correspondingly placed single boss in the second instar. Anterior spiracles placed as in second instar. Townsend (7) was unable to find these spiracles on his specimens. On the writer's they are very minute and escaped detection until a specimen in second ecdysis revealed their position.

Ventral armament of Segments IV to XI differs markedly from that of earlier stages. Instead of regular parallel rows of flat-topped, scale-like spines on the anterior part of each segment there are here irregular rows of sharp-pointed, posteriorly recurved spines. These rows number about 3 to 4 on Segment IV; 5 on V; 6 on VI; 7 on VII; 6 on VIII; 7 on IX; 7 on X; 4 on XI, 4 more widely spread on XII, and a few scattered spines on the anterior part of XIII. Entire posterior surface of proleg (Segment XIV) set with irregularly distributed spines.

Dorsal spines heavier but in fewer rows; 3 rows on IV, 3 on V and VI, about 4 on VII, VIII, IX, and X, 3 on XI, and on XII a few scattered spines anteriorly and a thin row posteriorly. Laterally on the posterior margins of Segments V to X a group of spines in a line dorsoventrally, a single row wide dorsally, two to three ventrally.

In addition to spines a few small pigmented dots appear behind the rows of spines as follows: on ventral surface between the lateral pair of bosses on Segments IV and V, an additional small group between the mid-ventral pair of bosses on Segments VI to XI with lateral groups becoming progressively larger and the dots larger and darker on each succeeding segment; lateral groups only on Segment XII; entire ventral surface of Segment XIII studded with these dots, a few on each side of XIV. On dorsal surface of Segments IV to XI at median base of each dorsal boss; a few dots mid-dorsally on IX, more on X; entire dorsal surface of XI, XII, and XIII heavily studded with larger dark dots.

Each segment from IV to XII provided with a series of boss-like peduncles arranged as follows on the otherwise naked posterior part of each segment.

One on each side of mid-ventral line, two close together on each side just below lateral ridge, one just above this ridge, and one on each side of the mid-dorsal line. Proleg with a boss on each side ventrally.

Posterior stigmata reniform, coarsely porose, dark brown, internal borders parallel, dorsal horn broader than ventral (Fig. 4), three foramina spaced equidistantly around dorsal and lateral external margin, a single well defined radially directed line setting off the dorsal horn, three additional incomplete lines, two dorsal and one ventral to the former. False stigma lightly sclerotized.

Cephalopharyngeal Skeleton

Similar in general to that of the second instar but relatively more massive throughout, from dorsal aspect hooks swollen basally rather than rugose; hypopharyngeal process more nearly equal to anterior transverse process in size; basal articulation with hypostomal sclerite broader. Hypostomal sclerite more elongate, anterior arms longer and swollen externally; basal transverse portion pierced by a foramen transmitting the common salivary duct. Basal sclerite relatively broader dorsoventrally and with lateral accessory process better developed. Dentate sclerite absent (Fig. 9).

Puparium

The only specimen (alcoholic) in the writer's possession measures 21 mm. by 10 mm. and is thought to represent the normal size. It developed from a fully mature larva. It is black in colour; narrowest at the anterior end and increasing gradually in diameter to the 10th or 11th segments. Spine rows are identical with those of the larva but non-spinous areas have shrivelled and lost form. There are numerous fine transverse wrinkles. The boss-like eminences are no longer present as such but their former sites can be detected in some instances because the epidermis there is not so closely wrinkled as elsewhere.

Summary and Comparisons

First instar larvae occur in the posterior part of the nasal passages from July to December or later. Larvae of this stage reach a length of about 3.5 mm.

Second instar larvae of *C. jellisoni* occur in the retropharyngeal recesses of the host from December or January until late May. They reach a length of 14 to 15 mm. before moulting.

Larvae of the third instar occupy the same site as the preceding stage until mature when they leave the host via the nasal passages to pupate in the ground. Mature larvae of this species reach a maximum length of 35 mm. This instar has been collected from late February to late June.

As regards external morphology, the first instar larvae are characterized by metapneustic respiratory system, slender mouth hooks with deeply incised basal sclerite, body form characterized by flattened marginal lappets, naked dorsum, and ventral surface provided with parallel rows of scale-like spines.

The second instar has the cephalopharyngeal apparatus distinctly different, dentate sclerites present, anterior and posterior stigmata—the latter triangular in outline and bearing coarse, irregularly shaped pores. Ventral surface with parallel rows of scale-like spines with castellate extremities, dorsal surface with sharp recurved spines.

Third instar has further modification of the cephalopharyngeal apparatus, lacks dentate sclerites, has both dorsal and ventral surfaces armed with stout, sharp, recurved spines. The posterior stigmata are large, reniform, coarsely porose and with certain other distinctive features; anterior stigmata minute.

Comparison with the life history of *C. auribarbis* (the nose bot of the red deer, *Cervus elaphus*, in Britain) as outlined by Cameron (1), reveals that *C. jellisoni* is closely similar. The mature second and third instar larvae, however, are smaller and there are certain other minor differences in morphology. Thus Cameron makes no mention of dorsal and ventral boss-like eminences on third instar of larvae *auribarbis* and he shows no mid-dorsal sulcus dividing the posterior fold of Segment II bilaterally. The sulcus forming the anterior boundary of the cervix is transverse and almost straight in *jellisoni*, strongly bowed anteriorly in *auribarbis*.

C. jellisoni apparently differs from *C. stimulator* Clk., *auribarbis* Mg., *ulrichi* Br., and *nasalis* L. (for which a key is provided by Cameron) in having the cervical area broader than long instead of in the reverse relationship.

The Deer Louse-fly, *Lipoptena depressa* Say

This parasite, known to hunters as the deer tick, occurs from southern British Columbia to the southern limits of the range of the coast deer in central California. It is among the most widespread and individually abundant external parasites of this deer in southern British Columbia, including Vancouver Island and the small adjacent islands.

None was present on three deer from Price Island and Porcher Island on the extremely humid part of the northwest coast but this is too small a number of specimens to establish a negative conclusion.

Spencer (6) discusses the distribution of the two named species of *Lipoptena* in western North America and finds that while *L. ferrisi* Beq. alone is present in the eastern part of the province (host *Odocoileus hemionus hemionus*) and *L. depressa* Say predominates on the coast (host *O. hemionus columbianus*) both species may be present at the same time on the latter host. He records both species from deer taken at the north end of Vancouver Island, B.C., Campbell River, Vancouver Island, B.C., and from Pemberton, B.C. The latter locality is in the area where the two races of deer come together and hybridize.

The writer has been unable to locate any published accounts of the life history of this fly and though he has made no attempt at experimental rearing and observation of adult flies he has kept notes on the observable features of the life history and has had a number of adults emerge in captivity from puparia taken in the field.

The following observations were made during the period October 1939 to August 1941 on southern Vancouver Island, and as climatic factors are no doubt involved at certain points in the life cycle the seasonal sequence may not be the same elsewhere in the range of the species.

In 1940 the first fly emerged in the rearing cages on May 26. This came from a premature puparium obtained on Jan. 31, 1940. This and all other puparia were kept in damp sand in a wooden diptera rearing cage out-of-doors throughout the entire experiment. The first fly to emerge from a normal puparium appeared on June 15, 1940. This puparium had been obtained on Dec. 19, 1939. The shorter quiescent period of the premature puparium was subsequently confirmed by further observations of such in comparison with normal puparia. Data on extent of quiescent periods are given in Table I.

Observations in the field confirmed this emergence date for 1941. On a deer taken at Goldstream Lake, June 13, 1940, were six newly emerged

TABLE I

PUPAL PERIOD OF *Lipoptena*

Date obtained	Date emerged	Elapsed time in days
Dec. 19, 1939	June 15, 1940	178
Dec. 19, 1939	July 7, 1940	200
Dec. 19, 1939	July 21, 1940	214
Jan. 31, 1940*	May 26, 1940	113
Feb. 2, 1940	July 4, 1940	152
Feb. 2, 1940	July 18, 1940	166
Feb. 22, 1940	Aug. 7, 1940	166
Mar. 19, 1940	June 16, 1940	89
Mar. 19, 1940	June 30, 1940	103
Mar. 19, 1940	July 30, 1940	133
Mar. 19, 1940	July 31, 1940	134
Mar. 19, 1940	Aug. 3, 1940	137
Mar. 19, 1940	Aug. 5, 1940	139
Mar. 19, 1940	Aug. 5, 1940	139
Mar. 19, 1940	Aug. 6, 1940	140
April 2, 1940	June 28, 1940	87
April 2, 1940	July 11, 1940	100
April 2, 1940	July 20, 1940	109
April 2, 1940	July 27, 1940	116
Aug. 16, 1940*	Sept. 28, 1940	43
Aug. 16, 1940*	Oct. 1, 1940	47
Aug. 16, 1940*	Oct. 5, 1940	51
Aug. 16, 1940*	Oct. 8, 1940	54
Aug. 16, 1940*	Oct. 11, 1940	57
Aug. 16, 1940*	Nov. 9, 1940	86
Aug. 16, 1940*	Nov. 29, 1940	106
Aug. 16, 1940*	Dec. 2, 1940	109

* *Premature puparia.*

imagos, three male and three female. All had just removed their wings but had not yet begun feeding. Deer taken on May 6, May 21, and June 4 bore only the last surviving individuals of the previous year's stock.

From the above date emergence continued throughout the summer and autumn. The latest sign of emergence noted in the field during the period of observation was Oct. 31, 1939. On this date 20% of 201 individuals taken from the head and neck of a buck shot on Goldstream summit were either winged or had but recently shed their wings. In captivity however emergence continued until Dec. 2, 1940, but it should be emphasized that the puparia contributing these late emergences in 1940 (Nov. 9, Nov. 29, and Dec. 2) were all premature puparia obtained Aug. 16 and might, under normal circumstances, have overwintered before metamorphosing.

In the spring and early summer it is possible to distinguish the survivors of last year's brood from the new brood, even pregnant females, by the more intense pigmentation of the older individuals,—they look brown and weather-beaten beside the new batch.

From midwinter the population of *Lipoptena* on the deer decreased gradually until in May it was reduced to countable numbers. A few old flies were still present among the newly emerged ones as late as July 14 and doubtfully on Aug. 16, but none was seen after that date. If November 15 be taken as an approximation of the date of the last fly emerging in the autumn of 1939 and June 15 as an approximation of the date of first emergence in 1939 it follows that the longevity of *Lipoptena* as an adult on the host animal lies between a maximum of 13 months and a minimum of eight months, the latter being the more probable figure.

On one occasion, Sept. 29, 1939, the writer observed two winged imagos copulating in flight. The pair landed on his clothing and were captured. There seem to be no published observations on the mating habits of this or related species that might help to indicate whether this is the normal procedure.

As has already been stated the first newly emerged adults were found on the host on June 13, 1940. On July 29, six of 50 young females taken were pregnant and contained young embryos of from 0.8 mm. to 1.1 mm. in length. The first mature or nearly mature larvae in individuals unmistakably of the new year's crop were obtained on Aug. 16, 1940. On this date, 19 pregnant females taken from a heavily infested host were far enough along to discharge the larvae they bore. Nine discharged black larvae and 10 white larvae that pupated after extrusion. Of these 10, three were near enough to normal pupation to produce normal black puparia. The remainder produced the undersized, brown puparia that have been referred to as premature. The evidence suggests that these undersized, brown puparia undergo a briefer quiescent period before emerging. One such pupa gave rise to an adult with shrivelled wings.

From the above data, and assuming, as is indicated as probable, that copulation takes place before reaching or shortly after reaching the host, the gestation period of *L. depressa* and/or *L. ferrisi* is approximately two months.

The mature larva assumes a black integument and is readily discernible within the abdomen of the female. The writer does not know how long the black larva is retained by the female but he has seen no indication that it is retained for any very extended period. It is unusual to find a female containing such a larva that has not a male clinging to her abdomen. Such associated pairs do not attempt copulation, the grip of the male on the female being quite different from that of amplexus; but the grip is none the less firm and is maintained until the two are forcibly parted. This might suggest that the presence of the male is in some way necessary for expulsion of the larva, but there is no further evidence to support this possibility. The writer has repeatedly observed successful delivery by unescorted females. Females in the earlier stages of pregnancy have never been found clasped by males. In fact the writer has no record of this clasping except in the presence of a mature larva *in utero*.

After leaving the body of the fly the larva assumes the definitive shape of the puparium and its integument hardens.

Cameron (2), speaking of the European species, *L. cervi*, refers to the puparia developing in the hair of the host. There is no indication of such a situation in *L. depressa*. The small, oval puparia are smooth and not sticky even when first deposited. Even if extruded on the dorsal part of the host they soon fall to the ground.

Studies on the distribution of this parasite upon the body of the host have revealed a strong tendency for the gravid females and their clasping mates to congregate on the lower surface of the body from the base of the neck to the abdomen. From such positions the puparia would almost invariably fall directly to the ground.

Death of the host interrupts the normal process. Most of the full term females deposit their larvae within 12 hr. However occasional individuals die without extruding the mature larva or with it partially expelled. Observed instances of this latter anomaly may have led Spencer (5) to conclude that the puparia were normally carried around attached to the female for a short period after extrusion. The process of larviposition is usually a rapid one, it may take as little as 30 sec. and seldom lasts more than three or four times that. As already stated, certain flies in which the white larvae are nearing maturity will expel these following the death of the host. Two types of puparia are formed from these larvae, depending on the advancement of their development. Large, mature larvae 3 mm. in length form normal black puparia. The smaller larvae either die without pupating or form undersized brown "premature" puparia.

Pupation frequently begins within 30 min. of extrusion in mature larvae. It involves a "rounding up" accompanied by the gradual appearance of black pigment on the dorsal and ventral surfaces, from which it slowly extends over the sides.

A white larva deposited at 8.30 A.M. Aug. 16, 1940, was showing black pigment dorsally and ventrally by 3.30 P.M. that day but pupation was not

complete until almost 11 P.M.,—a total elapsed time of a little over 14 hr. Further observations supported this schedule as a fair approximation of the usual condition. The shortest pupation time observed in 24 instances was six hours. This was a premature larva.

Apparently copulation may take place immediately following expulsion of the puparia, so that pregnancies succeed one another continuously during the life of the individual. On the other hand there are generally many females that are not obviously pregnant, though these have not been examined to determine the presence of very early larvae or of eggs. Of 56 *Lipoptena* taken from a deer on Jan. 6, 1940, 46 were copulating, and one female containing a black larva was also attended by a male.

If the calculated gestation period of two months is approximately accurate and pregnancies succeed one another with maximum rapidity, each female may produce from four to seven larvae during her life span.

Puparia were obtained in all months of the year except July. In that month the number of surviving adults is at a minimum and the new season's imagos have not yet produced their first offspring. Doubtless examination of a greater number of deer during July would fill this hiatus.

Puparia obtained at various times from mid-December, 1939, to mid-August, 1940, were placed in rearing cages and kept through to emergence. These cages were small wooden boxes with sliding lids. Each box was equipped with a screened ventilator at the back and a screened glass funnel in front. The puparia were placed on sand which was moistened once every week or two. The rearing cages were kept outside without protection from the elements. Table I details the result of this experiment. Though the material was not available for rearing it is obvious that puparia deposited in late October and November, if not those produced earlier, will winter over in the pupa stage and will thus have a pupal period of approaching 250 days. It is unfortunate that it was possible to obtain only a few specimens during May and June, and none in July. Those taken in May and June, 1939, failed to emerge but were viable and contained fully formed flies when they were accidentally destroyed a year later in June, 1940.

Rearing experiments so far conducted indicate a minimum pupal period of 43 days and this is thought to be unusually brief because of the immaturity of the pupa involved. The rather considerable individual variation in length of dormant period in puparia obtained on the same day and kept under identical conditions may be in part the result of the different ages of larvae prior to the forced deposition occasioned by death of the host.

Some simple experiments were conducted with newly emerged imagos to determine their tropistic responses at that time.

By placing these winged adults in a glass tube and manipulating the factors of light, heat, and orientation the following determinations were made.

These adults exhibit no positive thigmotropism, a strong contrast with the condition displayed by wingless adults removed from the host.

Negative geotropism was the strongest response exhibited. The insects always moved up, sometimes considerable wandering was involved but the end result was invariably the same, the insect came to rest at the upper end of the tube, even when this was the dark end.

Also displayed was a strong positive phototropism, though as already stated this was secondary to the negative geotropism.

Experiments to determine response to temperature were inconclusive. The flies did become more active in warm temperatures, as might be expected.

Both sexes are winged, and after emergence respond appropriately to light and gravity, seek and ascend vegetation, and take wing. The writer has been unable to ascertain the responses used in reaching the correct host. Winged individuals frequently alighted on his clothing and as far as the writer remembers this occurred for the most part when he was wearing a light brown sweater. Only two instances are recalled of these flies landing on dark clothing.

It is not known how long a time elapses between reaching a host and removal of the wings. During the period of emergence, from June to November, it is usual to find a small number of winged *Lipoptena* among the wingless flies on each parasitized deer. Others that have but recently shed the wings are also present. In these the body form is similar to that of the winged individuals and the stumps of the wings, raggedly removed at or near the humeral joint, are visible.

On hot summer days the newly wingless flies together with others that have been feeding and have their abdomens partially enlarged frequently move about over the surface of the pelt of the host in large numbers. A deer observed at close range on Aug. 16, 1940, had great numbers of these parasites moving over its face and visible on the legs. As feeding continues and pregnancy advances the females at least tend to congregate on the lower surface of the body and seem to favour the more thinly haired parts such as axilla and abdomen. Perhaps this is because these parts have also the thinnest hide.

Mature individuals on the host keep deep in the hair, and are seldom visible from the surface except on the white-haired abdomen and axilla.

Upon removal from the host winged and recently wingless individuals become very active. Both crawl rapidly up any object they can reach, the winged ones do not readily take wing but eventually do so. On the other hand flies that have fed for some time on the host display a strong positive thigmotropism that causes them to cling to any small object and to each other.

If a number of *Lipoptena* of varying ages are placed in a vial the older individuals will cling together in a mass at the bottom of the vial while the younger climb actively and as units.

In this fly it is possible to observe the gradual change from a free-living insect, with the tropistic responses adequate to bring about distribution with attendant discovery of host and mate, into a dependent parasite with the dominant responses centred upon clinging to the host.

Survival tests were conducted upon newly emerged flies and also upon sexually mature individuals removed from the host.

Seven newly emerged flies kept in the incubator at room temperature over damp sand survived for 72, 48, 52, 56, 60, 62, and 52 hr., the average survival time for this sample being 57 hr. Death of parasitic individuals began approximately 24 hr. after removal from the host and the last survivor was dead in six days. Of 201 flies removed from the head and neck of a buck shot Oct. 8, 1939, 90% were dead 48 hr. after death of the host. If left upon the host the louse flies apparently survive somewhat longer. A single living specimen has been observed on the host 11 days after the death of the deer and numbers have been taken alive after six and eight days.

The short survival time of the winged individuals must be a potent factor in limiting the population on areas where deer are scarce.

Infestations of this parasite reach a maximum in the late summer and early fall at the end of the period of emergence and gradually decrease to a minimum in May and June just prior to the emergence of the new season's winged adults. Heaviest infestations encountered have averaged two individuals per square inch of body surface, which on a deer with a body area of between 1000 and 1200 sq. in. indicates a population of *Lipoptena* of between 2000 and 2400 individuals.

Of 42 deer from southern Vancouver Island examined for this parasite only two were negative, one in December and one in January.

On several occasions the writer has found feeding punctures that he ascribed to this parasite. Such punctures were numerous on a heavily infested buck examined Feb. 13, 1940. No ticks were present on this deer and as no biting flies were at large it seems reasonable to assume that the observed punctures were the result of feeding activities of *Lipoptena*. Each puncture was the centre of a small red spot and in some cases a small amount of dried exudate was present at the site of the puncture.

Though this parasite is present in greatest numbers when the deer are at their optimum condition, nevertheless the irritation caused by the very heavy infestations must have considerable deleterious effect upon the host.

Though experimental evidence is lacking it would appear by inference that *Lipoptena* is not an agent in the life history of such helminth parasites as *Setaria* and *Onchocerca*. If *Lipoptena* were capable of acting as host to the larvae of these worms, and were capable of transmitting these larvae to the vertebrate host the observed population of these worms in individual deer should be much greater than it is.

Parasites of *Lipoptena* were not observed. It seems probable that shrews, white-footed mice, and seed-eating birds will destroy the puparium when they discover it. Possibly such predators comprise a major item in the control of this parasite.

Acknowledgments

Throughout the preparation of this report the writer has frequently availed himself of the advice of Professor G. J. Spencer of the Department of Zoology, University of British Columbia, Vancouver, B.C. This assistance is gratefully acknowledged. He is much indebted also to Dr. J. Bequaert, Harvard School of Tropical Medicine, for reading and criticizing the original manuscript.

The bulk of the data upon which this study is based was obtained while the author was a member of the staff of the British Columbia Provincial Museum, Victoria, B.C.

References

1. CAMERON, A. E. The nasal bot fly, *Cephenomyia auribarbis* Meigen (Diptera, Tachinidae), of the red deer *Cervus elaphus* L. Parasitology, 24 : 185-195. 1932.
2. CAMERON, A. E. Arthropod parasites of the red deer *Cervus elaphus* L. in Scotland. Proc. Roy. Phys. Soc. Edinburgh, 22 : 81-89. 1932.
3. HADWEN, S. Notes on the life history of *Oedemagena tarandi* L. and *Cephenomyia trompe* Modeer. J. Parasitol. 13 : 56-65. 1926.
4. JELLISON, W. L. *Cephenomyia pratti* (Diptera: Oestridae) reared from blacktailed deer. Proc. Helminthol. Soc. Wash. 2(2) : 69. 1935.
5. SPENCER, G. J. Ectoparasites of birds and mammals of British Columbia. II. A preliminary list of the Pupipara, louse flies. Proc. Entomol. Soc. British Columbia, No. 34: 39-45. 1938.
6. SPENCER, G. J. Ectoparasites of deer in British Columbia. Proc. Entomol. Soc. British Columbia, No. 35 : 15-19. 1939.
7. TOWNSEND, C. H. T. An undescribed American *Cephenemyia*. J. New York Entomol. Soc. 49 : 161-163. 1941.

Imperial Agricultural
New Delhi

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. JULY, 1943 NUMBER 7

MAIN ARTERIES IN THE NECK AND THORAX OF THE RHEA EMBRYO[1]

BY FRED H. GLENNY[2]

Abstract

A single specimen of a late rhea embryo was dissected and a diagram of the arteries in the neck and thorax prepared. Certain similarities and dissimilarities between the arterial patterns of the rhea, kiwi, and cassowary were noted. The left radix aortae was found to anastomose with the left pulmonary arch, with the subsequent degeneration of the left ductus botalli. The rhea is laevocarotidinae. The right internal carotid becomes functionally modified as do also the ducti carotici.

In April 1941, the writer had the opportunity of studying the arterial arrangement in the neck and thorax of a rhea embryo, *Rhea americana intermedia* (Linné), in the late embryonic stage. Although no definite incubation period could be established, the specimen obviously was very close to the hatching stage—most of the yolk mass being retained (*in corpore*). The embryo was one of the United States National Museum alcoholic specimens.

Routine dissection was carried out, and a diagram of the arrangement-pattern prepared. The arterial arrangement is set forth in the following observations and in Fig. 1.

In the late embryonic stage of the rhea, the left radix aortae (*5*) is found to maintain a proximal connection with the pulmonary (sixth aortic) arch (*6*). From studies on other birds, it is known that this connection results from anastomosis of the anterior portion of the radix (descending aortae) and the proximal portion of the sixth aortic arch (8, 9, 10). This is most readily observed in late embryonic stages of the Canada goose, black-billed cuckoo, Piciformes, and some of the Coraciiformes. After this anastomosis, the distal portion of the sixth aortic arch (ductus arteriosus/ductus botalli) atrophies and either disappears completely or fuses in part with the left radix aortae. Thus the left radix thereafter takes on the function of the ductus botalli (left side) until just prior to or for a very short period after hatching. (The fetal circulation is "short-circuited" by the ductus botalli which permits the flow of blood from the pulmonary arch to the radices aortae. At the same time, there is also pressure—from the blood in the radices aortae—exerted on the blood in the ductus botalli and this in turn exerts a pressure on

[1] *Manuscript received February 26, 1943.*
Contribution from the Department of Zoology, University of Toronto, Toronto, Ont.

[2] *Formerly Assistant, Department of Zoology, University of Toronto; now on active service with the Royal Canadian Army Medical Corps.*

the blood in the pulmonary artery. After obliteration of the left systemic arch, the posterior ramus of the radix aortae anastomoses with the left pulmonary arch, thereby maintaining in part a primitive type of vertebrate circulation. Subsequently the larger vessel (left radix aortae) takes over the

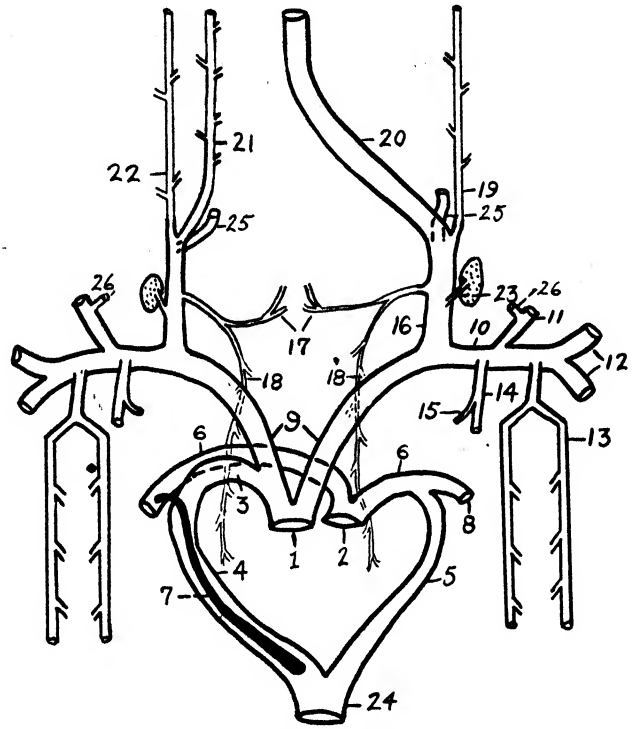


FIG. 1. *Diagrammatic representation of the arteries in the neck and thorax of Rhea americana intermedia, late embryo. Ventral view.*

KEY TO ABBREVIATIONS

(*1*) Aortic root; (*2*) pulmonary root; (*3*) systemic arch; (*4*) right radix aortae; (*5*) left radix aortae; (*6*) pulmonary arch (proximal portion); (*7*) ligamentum botalli (distal portion of sixth arch); (*8*) pulmonary artery; (*9*) innominate arteries; (*10*) subclavian artery; (*11*) axillary artery; (*12*) pectoral arteries; (*13*) intercostal artery; (*14*) coracoid major artery; (*15*) sternotracheal artery; (*16*) common carotid artery; (*17*) syringotracheal arteries; (*18*) ductus shawi; (*19*) left lateral superficial cervical artery; (*20*) left internal carotid (trunk) artery; (*21*) ascending-oesophageal artery; (*22*) right lateral superficial cervical artery; (*23*) thyroid artery; (*24*) dorsal aorta; (*25*) vertebral arteries; (*26*) coracoid minor artery.

normal function of the ductus botalli which, with reduced pressures from both proximal and distal ends, undergoes rapid degeneration. From observations on late embryonic and newly hatched stages of several widely separated species of birds, it appears that the rate and degree of atrophy of the left ductus botalli varies somewhat for the different families and orders of birds.

The factors involved in this are not clearly understood, but are probably based on phyletic relationships and possibly on certain unknown physiological factors of growth and development.)

Shortly after the left ductus botalli atrophies, the right ductus botalli begins to undergo a similar change, but, unlike its homologous structure, does not completely disappear. Instead, it remains as the ligamentum botalli (*7*) and in the adult is probably smaller than the ligamentum aortae.

The right systemic arch (*3*) alone remains as the functional arch of the adult and carries the blood by way of the right radix aortae (*4*) to the dorsal aorta (*24*).

The ductus caroticus becomes functionally modified as in other species of birds after losing its proximal attachment to the radices aortae[1] (Fig. 2). It remains as the ductus shawi (*18*) (3) and receives several branches from the trachea and syrinx (*17*), oesophagus, and other tissues dorsal to the heart[2]. Evidences of this have been found in many species of birds, but are most easily demonstrated in members of the Columbidae and in the hairy woodpecker, *Dryobates villosus villosus* L.

The left internal carotid artery (*20*) alone enters the hypapophysial canal and in this respect is not unlike many other species of birds such as the cassowary, kiwi, Piciformes, Passeriformes, and certain families of Coraciiformes (1, 2, 4–6, 8–11). The posterior or proximal length of the right internal carotid (*21*) remains as a superficial artery and appears to be functionally modified to serve as the ascending-oesophageal artery. This also appears to be the case in the kiwi (2, 4), Passeriformes (2, 5), Australian cassowary (6), the Piciformes, and certain families of the Coraciiformes (8, 9).

The innominate arteries (*9*) divide to form the subclavian (*10*) and the common carotid (*16*) arteries. The subclavians give off the coracoid major (*14*), axillary (*11*), intercostal (*13*), and two pectoral (*12*) arteries in order. The common carotids give off the ductus shawi (*18*) and the thyroid (*23*) arteries before dividing to form the internal carotid (trunk) (*20*), lateral superficial cervical (*19* and *22*), and vertebral (*25*) arteries.

The coracoid major sends off a smaller sternotracheal artery (*15*) to supply the sternotracheal muscle, while the axillary artery gives rise to the small coracoid minor artery (*26*). The intercostal artery bifurcates to form a ventral and a lateral branch.

By comparison, the general basic arrangement-pattern in the rhea is not entirely dissimilar to that of the kiwi and the Australian cassowary, but in some respects each is to be regarded as singular.

[1] "*. . . At the same time, the left ductus caroticus (dorsal portion of the radix aortae between the 3rd and 4th aortic arches) loses its proximal connection in the posterior ramus of the radix (due to the obliteration of the left 4th aortic arch) and becomes functionally modified thru subsequent anastomosis with several small arteries which form in situ and subsequently come to supply the oesophagus, trachea, syrinx, and other tissues of the thoracic cavity, dorsal to the heart.*" (*9*).

[2] "*. . . anastomoses of the ductus caroticus with syrinx, tracheal, and oesophageal arteries which form in situ to give rise to the ductus shawi (Glenny, 1940b, 1942a, 1942c) are quite common and are expected in birds.*" (*8*).

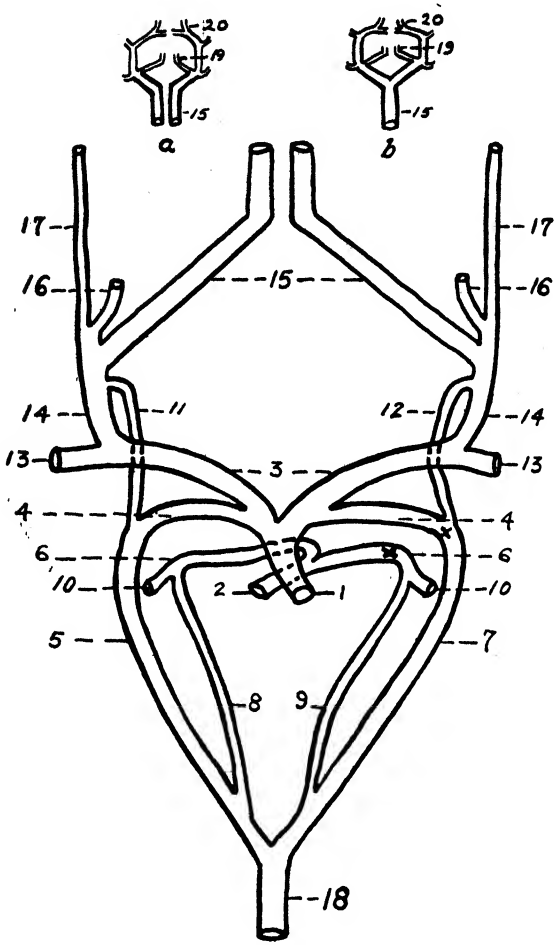


FIG. 2. *Diagrammatic representation of the aortic arches and associated main arteries in the neck and thorax of birds (to illustrate some of the changes that take place during middle and late embryogeny). Ventral view.*

Two small diagrams at top of page—cephalic branching of internal and external carotid arteries: (a) bicarotidinae normales; (b) laevocarotidinae.

KEY TO ABBREVIATIONS

(*1*) Aortic root; (*2*) pulmonary root; (*3*) innominate artery; (*4*) systemic (fourth aortic) arch; (*5*) right radix aortae (aorta descendens); (*6*) pulmonary (sixth aortic) arch; (*7*) left radix aortae; (*8*) right ductus botalli; (*9*) left ductus botalli; (*10*) pulmonary arteries; (*11*) right ductus caroticus; (*12*) left ductus caroticus; (*13*) arteria subclavia secunda; (*14*) ventral aorta; (*15*) internal carotid (trunk) artery (adult position); (*16*) vertebral arteries; (*17*) definitive or adult superficial cervical arteries; (*18*) dorsal aorta; (*19*) external carotid (maxillary) artery; (*20*) internal carotid artery; (*x*) marks the approximate position of the anastomosis between the left radix aortae and the left pulmonary arch (proximal portion).

Points of Similarity:

1. Left internal carotid (trunk) artery alone enters the hypapophysial canal—to carry the cephalic blood supply.

2. Right internal carotid (trunk) artery becomes functionally modified as the primary ascending-oesophageal artery.

3. Left radix aortae remains as a prominent ligamentous vestige of the embryonic vessel—ligamentum aortae.

4. Ductus caroticus is functionally modified to form the ductus shawi—several smaller vessels which form *in situ* anastomose with the ductus caroticus prior to or just after it loses its proximal connection with the systemic arch.

Points of Dissimilarity:

1. Major branches of the subclavian arteries vary in relative position and in supply (in the kiwi and cassowary) from the general avian pattern.

2. Secondary vessels differ in points of origin, number, and supply.

3. Relative position (origin) of the internal carotid (trunk), vertebral, and superficial cervical arteries is different in each of the three species.

4. In *Casuarius australis* the right cervico-intercostal artery sends off a dorsal intercostal artery and several pairs of short segmentally arranged dorsal intercostal and cervical twigs before becoming the right vertebral artery.

References

1. BEDDARD, F. E. The structure and classification of birds. Longmans, Green, and Company, London. 1898.
2. GARROD, A. H. On the carotid arteries of birds. Proc. Zool. Soc. London, 457-472. 1873.
3. GLENNY, F. H. The main arteries in the region of the heart of three species of doves. Bull. Fan Memorial Inst. Biol., Zool. Ser., 10 : 271-278. 1940.
4. GLENNY, F. H. Arteries in the heart region of the kiwi. Auk, 59 : 225-228. 1942.
5. GLENNY, F. H. A systematic study of the main arteries in the region of the heart—Aves III, Fringillidae, Part 1. Ohio J. Sci. 42 : 84-90. 1942.
6. GLENNY, F. H. Main arteries in the region of the neck and thorax of the Australian cassowary. Can. J. Research, D, 20 : 363-367. 1942.
7. GLENNY, F. H. A systematic study of the main arteries in the region of the heart—Aves VI, Trogoniformes, Part 1. Auk, 60 : 235-239. 1943.
8. GLENNY, F. H. A systematic study of the main arteries in the region of the heart—Aves IV, Piciformes, Part 1. Proc. Zool. Soc. London. In press. 1943.
9. GLENNY, F. H. A systematic study of the main arteries in the region of the heart—Aves VII, Coraciiformes, Part 1. In preparation.
10. GLENNY, F. H. A systematic study of the main arteries in the region of the heart—Aves VIII, Anseriformes, Part 1. In preparation.
11. GLENNY, F. H. A systematic study of the main arteries in the region of the heart—Aves IX, Coliiformes, Part 1. In preparation.

DRIED WHOLE EGG POWDER

II. EFFECT OF HEAT TREATMENT ON QUALITY[1]

By W. Harold White[2] and M. W. Thistle[2]

Abstract

The effect on quality of heating dried egg powders from two Canadian plants at temperatures ranging from 26.7° to 60.0° C. for periods of from three hours to seven days was investigated. Quality of the treated powders was assessed by determination of the fluorescence, potassium chloride, and water values and the pH and refractive indices of the potassium chloride and aqueous extracts.

The rate of deterioration of quality was on the average usually greatest on heating for one day at temperatures of 43.3° C. and higher. However, some change was observed even at 26.7° C. after one day. The powders from the two plants behaved similarly. Interpretation of the results in terms of the rate at which egg powder should be cooled after drying indicated that a temperature of 26.7° C. or less should be attained within three hours if deterioration in quality is to be prevented.

Introduction

Until recently dried whole egg powder was usually considered to be a relatively stable food product. Accordingly little attention has been given to the temperature conditions to which dried egg is exposed after its manufacture. However, experience with other foodstuffs has shown that temperature is usually one of the prime factors determining the rate of deterioration. Examination indicated that deterioration of dried egg might result either from the relatively high temperatures of the powder when removed from the drier and the extended period required for it to cool in commercial packages or from subjection to unfavourable temperature conditions during storage and transportation. The present paper deals with the effect on quality of temperatures comparable with those to which dried whole egg powder may be subjected on removal from commercial driers. Studies of the effect of storage temperature and other factors, such as the moisture content and gas packing, will be described in subsequent papers.

Material and Procedure

Egg powder was obtained from two plants, selected to represent the two main types of drying practice used in Canada. In Plant I, Grades B and C storage eggs were dried in a box type drier at an inlet temperature of 149° C. (300° F.) and an outlet temperature of about 63° C. (145° F.). In Plant II, Grades B and C storage eggs were dried in a cone type drier at approximately the same temperatures as noted above.

[1] *Manuscript received April 29, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Material selected from a thesis presented by one of us (W.H.W.) to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as paper No. 90 of the Canadian Committee on Food Preservation and as N.R.C. No. 1125.

[2] *Biochemist, Food Investigations.*

Immediately upon removal from the drier the powder was placed in rubber latex bags, and cooled to a temperature of 0° C. The powder was shipped from the plants in special containers at a temperature of 0° C. Upon arrival at the laboratory, 100-gm. samples were sealed in tin cans in a room at 4° C. (40° F.). It was considered that these precautions were sufficient to prevent any change in the quality from the time of collection of the powder to its treatment in the laboratory.

The specially collected powders from each of the two plants were heated rapidly to, and maintained at, temperatures of 26.7°, 35.0°, 43.3°, 51.7°, and 60.0° C. (80°, 95°, 110°, 125°, and 140° F.). Samples were removed for analysis after heating for 3, 6, and 12 hours and one, two, three, four, five, six, and seven days, and immediately cooled to 0° C. The selection of temperatures was based on the following considerations: 35.0°, 43.3°, and 51.7° C. represented approximately the minimum, average, and maximum temperatures of the powders on leaving the driers, as observed in Canadian plants; 26.7° C. was considered to be a temperature to which powder could be cooled without excessive practical difficulty; and 60.0° C. was selected to determine the effect of an abnormally high temperature on the quality of dried egg.

The quality of the powder after various heat treatments was assessed by determination in duplicate of the fluorescence, water, and potassium chloride values. The methods employed have been described previously (2, 3). The fluorescence value gives a measure of the overall quality of dried egg and is related to flavour quality (2). The potassium chloride and, to a lesser extent, the water values are related to overall quality, and also indicate the solubility of egg powder (3).

In addition, the pH and refractive indices of the potassium chloride and aqueous extracts were determined, since it was considered that they might bear some relation to quality. Measurements of the pH were made at 25° C. on residual solutions from the potassium chloride and water value determinations with a Beckman pH meter, using a glass electrode and a saturated calomel half-cell. Refractive indices of the same solutions were measured in an Abbé refractometer at 25° C.

Results

Analyses of Variance

The relative importance of temperature, time of heating, and source of the egg powder in affecting the quality was determined by means of analyses of variance. The results (Table I) show that temperature had the greatest effect on quality, as assessed by all the measurements. However, differences that were attributable to variations in the time of heating and the plant from which the egg powder was obtained usually attained statistical significance.

Mean values for each variable, as calculated over all others for the whole experiment, are given for each measurement in Table II. It is to be noted that the data obtained for the refractive indices of the potassium chloride

TABLE I

ANALYSES OF VARIANCE FOR THE EFFECT OF HEAT TREATMENT ON THE QUALITY OF DRIED EGG POWDERS, AS ASSESSED BY VARIOUS PHYSICAL AND CHEMICAL MEASUREMENTS

Source of variance	D.f.	Mean square						
		Fluorescence value	KCl value	Water value	pH of KCl extract	pH of aqueous extract	Refractometric value of KCl extract	Refractometric value of aqueous extract
Temperature	4	65,907**	16,388**	12,741**	8.6530**	5.1132**	2284.0**	1544.4**
Time	10	17,800**	3974.4**	1834.6**	2.6008**	0.9703**	489.23**	337.02**
Plants	1	5650.4**	7401.9**	1196.2**	1.3904**	0.3496**	868.04**	29.823
Temperature × time	40	3390.1**	706.13**	657.82**	0.3113**	0.2929**	113.93**	83.763**
Plants × temperature	4	459.21**	61.777	300.02*	0.0342	0.0296	5.3818	53.061*
Plants × time	10	195.53**	22.955	220.78*	0.0176	0.0608	9.7209	41.473**
Residual	40	60.811	34.042	87.718	0.0133	0.0152	9.6118	14.324
Duplicate error	110	1.5961	0.8703	1.4787	0.0021	0.0024	1.9227	3.2318

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

TABLE II

MEAN VALUES FOR THE EFFECT OF HEAT TREATMENT ON THE QUALITY OF DRIED EGG POWDERS, AS ASSESSED BY VARIOUS PHYSICAL AND CHEMICAL MEASUREMENTS

Factor	Fluorescence value[1]	KCl value[1]	Water value[1]	pH of KCl extract[1]	pH of aqueous extract[1]	Refractometric value of KCl extract[1]	Refractometric value of aqueous extract[1]
Temperature, ° C.							
26.7	16.5	78.5	74.8	8.39	8.44	31.0	28.5
35.0	22.3	76.6	77.5	8.28	8.39	29.5	29.7
43.3	36.8	60.3	70.2	8.01	8.23	24.0	27.3
51.7	82.2	42.9	48.7	7.61	7.90	17.6	20.2
60.0	104.0	35.7	39.4	7.34	7.64	14.5	16.0
Necessary difference[2]	3.4	2.5	4.0	0.05	0.05	1.3	1.6
Time, days							
0	13.8	76.4	69.9	8.45	8.40	29.2	30.8
0.13	14.3	80.4	72.1	8.38	8.36	30.0	28.0
0.25	21.5	76.6	75.7	8.36	8.37	30.4	28.1
0.50	32.3	68.1	75.5	8.19	8.30	25.0	28.4
1	42.1	59.0	61.8	8.00	8.25	25.5	25.2
2	49.0	53.8	59.1	7.83	8.07	21.9	23.6
3	62.8	49.2	58.6	7.67	8.04	20.0	23.6
4	76.3	48.9	54.6	7.64	7.95	20.6	21.4
5	86.6	45.7	51.9	7.57	7.85	18.0	20.4
6	88.6	44.8	53.8	7.56	7.90	18.0	20.4
7	88.8	43.8	50.3	7.57	7.83	17.9	17.8
Necessary difference[2]	5.0	3.7	6.0	0.07	0.08	2.0	2.4
Plant							
I	47.3	64.6	64.5	8.01	8.16	25.3	24.7
II	57.4	53.0	59.8	7.85	8.08	21.3	24.0

[1] *Mean values for all other conditions over the whole experiment.*
[2] *Necessary difference required to exceed 5% level of statistical significance.*

and aqueous extracts have been reduced for the sake of convenience to the form of "refractometric values", i.e. (refractive index of extract − refractive index of solvent) × 10^4. In general, differences between temperatures were usually least between 26.7° and 35.0° C. and greatest between 43.3° and 51.7° C. The greatest change with time occurred usually during the first day. The powder from Plant I deteriorated at a slower rate than that from Plant II. This difference is considered to be due primarily to the fact that the powder from Plant I contained 3.5% moisture while that of Plant II contained 5.6%. It has been found that the stability of egg powder decreases with increase in the moisture content. Details of this investigation are to be published in the near future.

Details of the changes in the fluorescence and the potassium chloride values, and the pH and refractometric values of the potassium chloride extracts are graphically illustrated in Figs. 1 to 4, respectively. Curves for the corresponding measurements on aqueous extracts were in general similar to those given.

Fluorescence Value

An increase in either the temperature or time of heating caused an increase in the fluorescence values (Fig. 1). The rate of formation of fluorescence materials was relatively great at all temperatures above 26.7° C. However, even at this temperature, deterioration in quality occurred if the powder was heated for more than three hours.

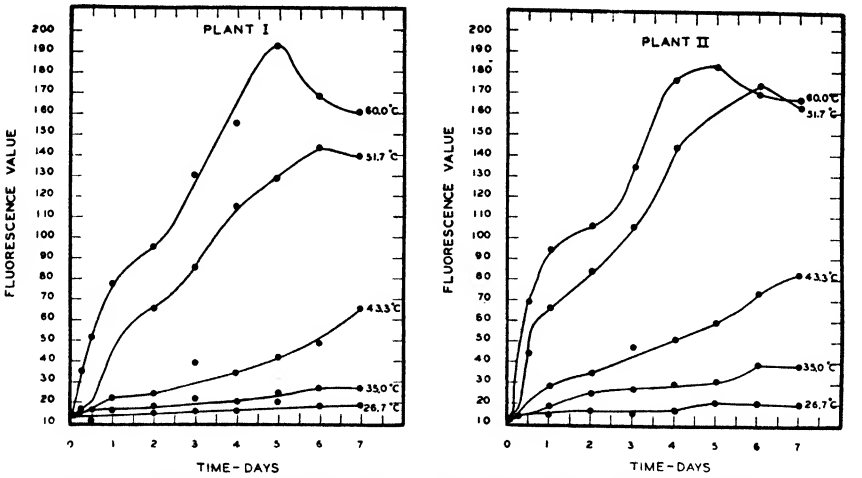


FIG. 1. *Effect of heat treatment on the fluorescence values of dried egg powders.*

The results of a recent investigation indicate that the fluorescent materials are formed by hydrolysis of the protein fraction of egg powder (1). It is of some interest to examine the present data with respect to the nature of the reactions producing the fluorescent materials. The general conformity of the curves (Fig. 1) for the two plants suggests that similar types of reactions

were occurring in both powders regardless of their method of preparation. At 51.7° and 60.0° C. there is some evidence that two or more reactions yielded fluorescent end products. Since there was some indication that the fluorescence values decreased after reaching a maximum, it would seem that at these two temperatures the reactions had reached completion, and that fluorescent materials were being thermally decomposed.

Potassium Chloride and Water Values

The potassium chloride (Fig. 2) and water values of the powders decreased on heating, indicating thermal decomposition of the fat–protein complex and denaturation of the egg protein. The most marked decreases were observed at 51.7° and 60.0° C. on heating for one day. Subsequent changes

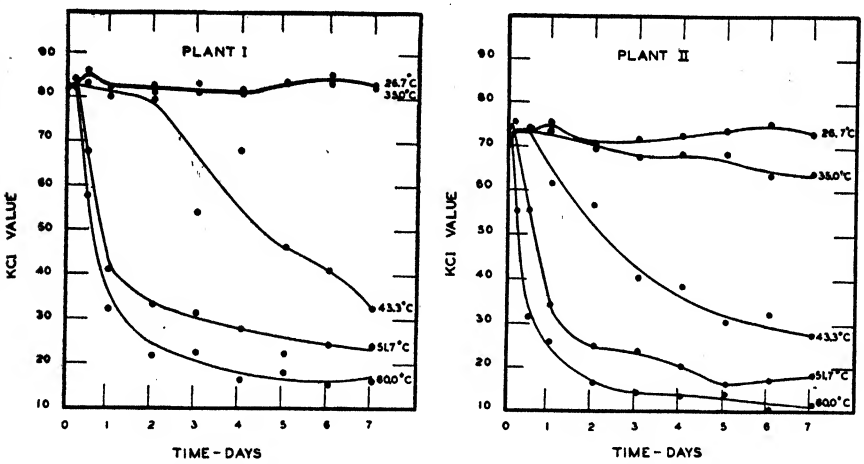


FIG. 2. *Effect of heat treatment on the potassium chloride values of dried egg powders.*

at these temperatures were relatively slow, indicating that the denaturation reactions were approaching completion. The decreases observed at 26.7° and 35.0° C. were relatively small and approximately of the same order of magnitude. The reason for the slight increase in potassium chloride and water values for the shorter heating periods is unknown. However, it may be that the nature of the fat–protein complex is altered, thereby permitting more fat to pass through the filter paper.

In actual magnitude the water values were lower initially than the potassium chloride values, and changed less during the experiment. This behaviour suggests that not only could more of the egg powder be dissolved or dispersed in 10% potassium chloride solution than in water, but that these materials were more unstable thermally. Moreover, the water values were rather variable and decreased appreciably with increase in the time of heating only at temperatures of 43.3° C. and higher. Thus, the potassium chloride value is a more sensitive and precise measurement of quality than the water value.

In general the potassium chloride and water values were less sensitive to the effects of heat treatment than the fluorescence value.

pH of Potassium Chloride and Aqueous Extracts

The pH of the potassium chloride (Fig. 3) and aqueous extracts decreased as the temperature and time of heating were increased, indicating that the thermal decomposition products were acidic in character. The greatest changes usually occurred during the first day and at temperatures of 51.7° and 60.0° C. The data for the pH of aqueous extracts were more variable than those for the potassium chloride extracts and the magnitude of the changes was smaller. This is in agreement with previous observations on potassium chloride and water values.

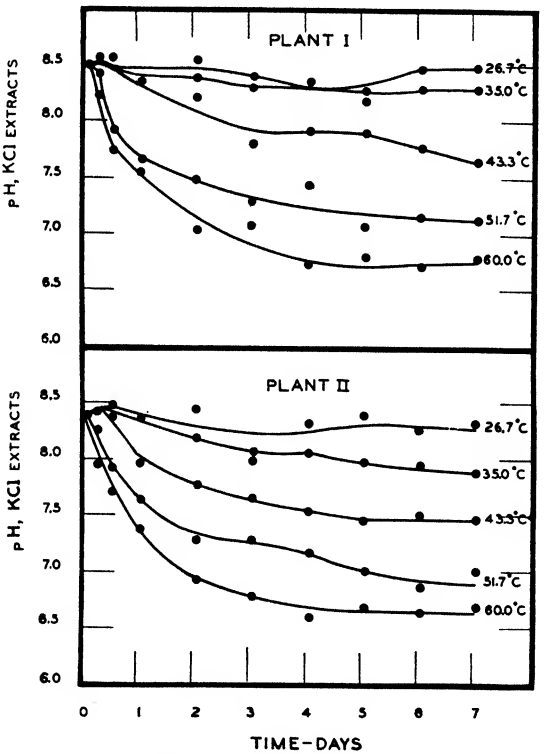


FIG. 3. *Effect of heat treatment on the pH of potassium chloride extracts of dried egg powders.*

Refractometric Values of Potassium Chloride and Aqueous Extracts

The refractometric values of the potassium chloride and aqueous extracts were somewhat small because of the relatively dilute extract of egg powder used, and are consequently subject to a relatively large experimental error in

measurement with the Abbé refractometer. In spite of this the determination was sufficiently sensitive to detect changes similar to those observed for the other measurements on these extracts (cf. Figs. 2, 3, and 4). An increase in either the temperature or time of heating caused a decrease in the refractometric values for both plants. The values for Plant I were usually higher at all temperatures and times of heating.

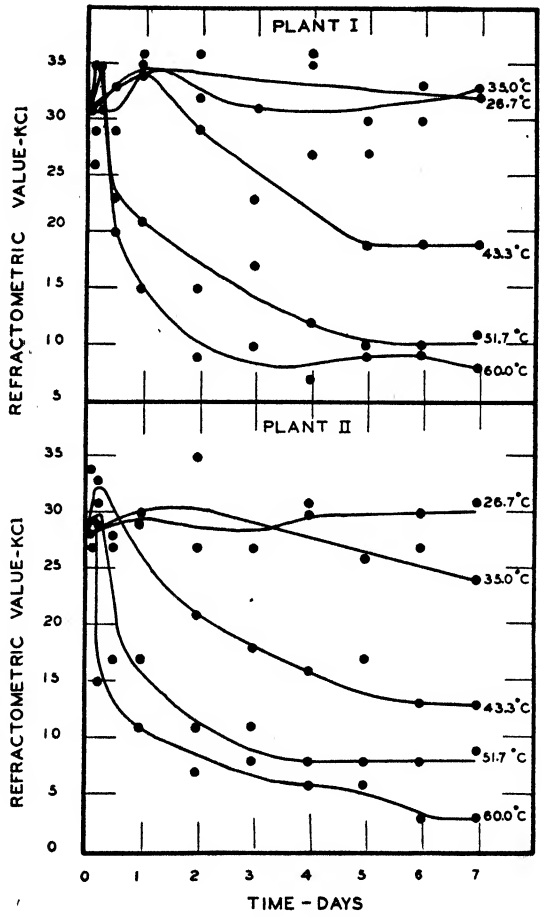


FIG. 4. *Effect of heat treatment on the refractometric values of potassium chloride extracts of dried egg powders.*

Interrelation of Methods for Assessing Quality

It is apparent from the preceding discussion that the methods employed for assessing quality were more or less related. The degree of interrelation was determined by the computation of simple coefficients of correlation. Data were used for only those samples that corresponded to egg powder of

suitable quality for export by present Canadian standards, i.e. a fluorescence value of 52 or lower and a potassium chloride value of 40 or higher. Even within these limits the coefficients are possibly unsuitable for prediction purposes because of a higher proportion of low grade material than would be obtained in Canadian egg powders prepared for export. The fluorescence and potassium chloride values, and the pH and refractive index of potassium chloride extracts would appear to be the most suitable of the methods studied for estimating deterioration in the quality of egg powder as a result of adverse heat treatment (Table III).

TABLE III

COEFFICIENTS OF CORRELATION BETWEEN VARIOUS MEASUREMENTS OF QUALITY OF DRIED WHOLE EGG POWDER

Methods	Methods					
	pH of water extract	pH of KCl extract	Refractometric value of aqueous extract	Refractometric value of KCl extract	Water value	KCl value
Fluorescence	−0.62**	−0.86**	−0.24*	−0.78**	−0.14	−0.84**
KCl value	0.66**	0.88**	0.26*	0.84**	0.22	—
Water value	0.33**	0.16	0.64**	0.11	—	—
Refractometric value of KCl extract	0.69**	0.81**	0.17	—	—	—
Refractometric value of aqueous extract	0.45**	0.22	—	—	—	—
pH of KCl extract	0.62**	—	—	—	—	—

* *Exceeds 5% level of statistical significance for the 68 degrees of freedom available.*
** *Exceeds 1% level of statistical significance for the 68 degrees of freedom available.*

Discussion

While the investigation was concerned with the effect of heat treatment on the quality of egg powder, the results can be interpreted in terms of the rate and temperature to which dried egg should be cooled as it comes from the drier. Of the conditions studied, deterioration in quality occurred if powder was heated at temperatures of 35.0° C. and higher for three hours, whereas there was little change at 26.7° C. in this period. Hence, it may reasonably be concluded that dried egg should be cooled to 26.7° C. or lower within three hours after removal from the drier.

This conclusion has been confirmed by practical tests in Canadian egg drying plants. In these the quality of promptly cooled powder was compared with that of the same powder treated by the normal practices of the particular plant. Results obtained for two plants are given in Table IV. The effectiveness of rapid cooling in maintaining quality is obvious.

The marked thermal instability of dried whole egg powder, as demonstrated in the experimental investigation and confirmed in commercial practice, has

TABLE IV

COMMERCIAL TESTS ON THE EFFECT OF RAPID COOLING ON THE QUALITY OF CANADIAN DRIED EGG POWDER

Plant	Normal cooling		Rapid cooling	
	Fluorescence value	Predicted flavour score[1]	Fluorescence value	Predicted flavour score[1]
A	29.2	7.1	14.8	9.6
B	25.2	7.7	13.3	9.9

[1] *Computed from the equation given by Pearce and Thistle* (2).

led to the adoption of a regulation requiring that all Canadian egg powders prepared for export to England be cooled to a temperature of 26.7° C. or lower within one hour from the time of their formation. It is considered that rigid adherence to this regulation will materially raise the general level of quality of Canadian dried egg powder.

Acknowledgments

The authors wish to express their thanks to Mr. W. D. B. Reid for making the statistical computations and to Mrs. Margaret Reid and Mr. D. A. Fletcher for their technical assistance.

References

1. PEARCE, J. A. Can. J. Research, D, 21 : 98-107. 1943.
2. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20 : 276-282. 1942.
3. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.

DRIED WHOLE EGG POWDER

III. A REFRACTOMETRIC METHOD FOR THE DETERMINATION OF SOLUBILITY[1]

By W. Harold White[2] and G. A. Grant[3]

Abstract

The refractometric determination of the solubility of dried, whole egg powder was found to be affected by the method of defatting, the nature of the fat and protein solvents, the ratio of protein solvent to powder, the method of equilibration, and time of extraction. These factors were standardized by defatting egg powder with petroleum ether and extracting for a period of two hours with a 5% solution of sodium chloride. The refractometric value of the extract was determined at 25° C. using an Abbé refractometer.

The refractometric value was linearly related to the content of water soluble nitrogen of whole powder and to the potassium chloride value of defatted powder. A curvilinear relation was obtained with the content of crude albumin nitrogen and with the potassium chloride value of whole egg powder.

Introduction

Egg powders of edible quality may be divided into two groups, namely, those fit for consumption as egg dishes, and those satisfactory only for use by bakers or other food manufacturers requiring a source of edible, soluble protein for their products. Hence, solubility is a useful criterion for grading egg powders. The present paper describes a rapid and convenient refractometric method for determining the solubility of egg powder.

None of the known methods for the determination of the solubility of egg powder are entirely satisfactory. Methods involving the Kjeldahl determination of nitrogen (2, p. 309, 4) are somewhat time consuming, and depend on obtaining a clear extract from the egg powder. For the most part, the potassium chloride value (6) gives a measure of overall quality rather than of solubility alone. A method based on the determination of the volume of the water soluble, heat-coagulable protein (5) was considered to be somewhat insensitive. In these circumstances attention was given to other possible procedures for determining solubility. Of these, refractometric methods have been successfully used for the determination of the solids content of shell eggs (1, 3, 7). The results of a preliminary study indicated that they offered possibilities of suitable adaptation to dried egg (8).

[1] *Manuscript received April 28, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Material selected from a thesis presented by one of us (W.H.W.) to the Faculty of Graduate Studies and Research, McGill University, Montreal, Que., in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as Paper No. 91 of the Canadian Committee on Food Preservation and as N.R.C. No. 1126.

[2] *Biochemist, Food Investigations.*

[3] *Laboratory Assistant, Food Investigations.*

Procedure and Results

Fat Extraction

A preliminary study was made of the relative suitability of using whole or defatted egg powder for the determination. For this purpose 1 gm. samples of whole egg powder were extracted with 5 ml. of a 5% solution of sodium chloride for 30 min. No marked separation of the liquid and solid phases occurred except in powders of poor quality. This made accurate determination of the refractive index of the extract in the Abbé refractometer difficult. Removal of the fat prior to extraction gave a much clearer extract, and it appeared that extraction of the protein would be facilitated by the removal of the fat fraction. Attempts to extract the fat and soluble protein with mixed solvents in one step were unsuccessful, indicating that the two treatments should be made separately.

The effect of defatting was investigated on four samples of egg powder known to differ in their contents of soluble protein. Four grams of each powder were defatted by manual shaking at room temperature with four 50 ml. portions of petroleum ether (boiling range 30 to 60° C.), and filtered each time through a No. 1 Whatman filter paper. The defatted powders were allowed to stand about 30 min. at room temperature to permit the evaporation of residual solvent. Both the whole and defatted powders were extracted by shaking 1.00 gm. with 5 ml. of 10% potassium chloride solution at three 10 min. intervals. The refractive indices of a small portion of the extracts were determined at 25° C. in an Abbé refractometer.

The results, given in Table I, are expressed as "refractometric values", that is (refractive index of extract − refractive index of solvent) × 10^4. With the exception of one sample the refractometric values for defatted powder were higher than those for whole powder. This was to be expected because of the greater amount of protein material present in defatted powder. Thus, in addition to giving extracts that can be more easily read in the Abbé refractometer, the use of defatted powder permits a relatively more accurate and precise determination of solubility by virtue of the larger readings obtained.

TABLE I

EFFECT OF DEFATTING AND OF THE SOLVENT USED FOR EXTRACTION OF PROTEIN ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Whole powder		Defatted powder		
1 gm. samples		0.6 gm. samples	1 gm. samples	
5% NaCl	10% KCl	10% KCl	5% NaCl	10% KCl
225	220	150	229	206
192	177	147	217	209
120	112	115	176	170
53	49	49	82	76

* *Expressed as (refractive index of extract − refractive index of protein solvent) × 10^4 at 25° C.*

The difference in refractometric values of 1 gm. of whole and defatted powder was smaller than expected for the two samples of prime quality. This suggested that a portion of the egg powder that is normally capable of being dissolved in 10% potassium chloride solution was removed by defatting. Accordingly refractometric values were determined on 0.6 gm. of defatted powder, an amount approximately equivalent to the non-lipid fraction of 1 gm. of whole powder. The values obtained (Table I) were smaller than those for an equivalent amount of whole powder for the first two samples, and approximately the same for the two heat denatured samples. This suggests the presence in egg powder of a fat–protein complex that can be dispersed or dissolved in 10% potassium chloride solution or petroleum ether, and that is decomposed by heat (6).

As a result of the above observations, the relative suitability of various solvents for defatting egg powder was investigated. For this purpose, four samples of egg powder were defatted by the method described previously with each of the solvents, petroleum ether, acetone, chloroform, and benzene. One gram samples of the defatted powder were shaken with 5 ml. of a 5% solution of sodium chloride for two hours, and the refractive index measured in the Abbé refractometer. A 5% sodium chloride solution was used on the basis of the results of a simultaneous study on protein solvents.

The refractometric values (Table II) for samples defatted with petroleum ether or acetone were approximately of the same magnitude, and lower than those for powders treated with chloroform or benzene. The use of petroleum ether or chloroform gave slightly more precise results. Petroleum ether was selected as the most suitable solvent of those studied on the basis of such considerations as its ease of removal from the defatted powder, and its relatively non-toxic character. Moreover, since the test was to be used for routine quality control, it was desirable to avoid the tedious filtering procedure. The use of petroleum ether rather than chloroform made centrifuging possible.

TABLE II

EFFECT OF SOLVENT USED FOR DEFATTING ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Petroleum ether	Acetone	Chloroform	Benzene
250	251	255	258
229	225	228	239
214	212	222	226
144	143	163	156

* *As defined in Table I.*

The suitability of separating the powder and solvent by centrifuging was investigated. Four-gram portions of each of four different egg powders were weighed into 50 ml. centrifuge tubes, 40 ml. of petroleum ether added, the

mixture stirred, then centrifuged for approximately five minutes, and the liquid decanted. This procedure was repeated three times. The same four samples were defatted by the filtering procedure previously described. Refractometric values were determined, using 5% sodium chloride solution.

Refractometric values obtained for samples defatted by these two procedures are given in Table III. The centrifuging method was selected as being the more suitable since it was more rapid and convenient experimentally, and moreover, gave slightly higher results. The reason for this latter behaviour is not apparent. It was subsequently considered that the number of fat extractions could be reduced to two without materially affecting the results.

TABLE III

EFFECT OF METHOD OF DEFATTING ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Method of defatting		Method of defatting	
Filtering	Centrifuging	Filtering	Centrifuging
257	265	221	229
233	244	143	146

* *As defined in Table I.*

Protein Extraction

The relative suitability of two protein solvents, namely, a 5% solution of sodium chloride and a 10% solution of potassium chloride (4) was studied on four different samples of whole and defatted powder. The results obtained are given in Table I. The refractometric values with the sodium chloride solution were higher than with the potassium chloride solution for both whole and defatted powder. Moreover, the sodium-chloride–soluble components of defatted powder appeared to be somewhat more prone to heat denaturation since the range of values between samples of good and poor quality was greater. A 5% solution of sodium chloride was accordingly selected as being the more suitable solvent. Since the extract obtained with this solvent from defatted powder gave sufficient sensitivity to the determination as a whole, it appeared that further comparison of protein solvents was unnecessary.

The effect of extraction with various amounts of sodium chloride solution on the refractometric value was studied on four samples of egg powder. To 1 gm. of defatted egg powder were added 5, 10, 15, or 25 ml. of 5% sodium chloride solution. The samples were mechanically mixed for two hours and the refractometric values determined at 25° C. in the Abbé refractometer. The results, given in Table IV, show that as the ratio of solvent to powder is increased, the refractometric values decrease. Since the samples treated with 5 ml. of solvent gave the highest values and the greatest difference

TABLE IV

EFFECT OF VOLUME OF SOLVENT USED FOR EXTRACTION OF PROTEIN ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Volume of 5% NaCl solution, ml.			
5	10	15	25
260	130	82	50
236	123	80	47
243	118	79	42
102	40	23	20

* *As defined in Table I.*

between samples of poor and good quality, this volume was selected as being the most suitable of those studied.

One of the most important steps in the determination of solubility of egg powder is the method employed for equilibrating the dried egg with the solvent. The relative effectiveness of a number of possible methods was studied. In the first of these investigations four samples of defatted egg powder, to which 5 ml. of a 5% solution of sodium chloride had been added, were shaken both by hand and in a mechanical shaking machine. When shaken by hand the test-tubes containing the samples were inverted 10 times at four equal intervals during the periods studied.

Refractometric values of the four samples were: 253, 233, 185, and 89 with mechanical shaking, and 235, 229, 179, and 90 with manual shaking. It would appear that mechanical shaking was more effective than the manual method employed. It was found also that solution of the protein was aided by the presence of glass beads in the test-tube during mechanical shaking. However, visual observation indicated that more effective mixing of the solvent and powder would be desirable.

The apparatus shown in Fig. 1 was found to give thorough mixing of the solvent and powder. When the test-tube is shaken at a rate of approximately 300 impulses per minute, the glass rod fastened to the lower end of the flexible rubber tubing moves back and forth rapidly in the tube, thoroughly mixing the contents. It was found that shaking at higher speeds gave an extract that was difficult to read in the Abbé refractometer.

The time required for solution was studied next, using four samples of egg powder. After defatting by the centrifuge method described previously, 1 gm. samples were weighed into test-tubes, 5 ml. of 5% sodium chloride solution added, and the samples mixed by the method described above. Refractive indices of the extracts were determined after shaking for periods of 0.5, 1, 2, and 5 hours.

The results (Table V) show that equilibrium had for the most part been reached after shaking for two hours, although there was some indication that

complete solution of the soluble constituents of high quality powder may require periods of five hours or longer. However, since the differences were small, prolongation of the extraction period beyond two hours usually did not appear to be warranted.

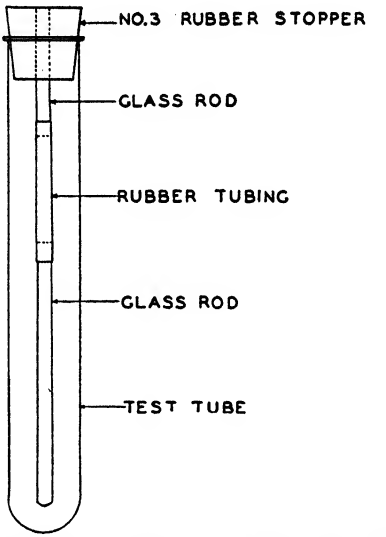


FIG. 1. *Diagram of apparatus employed for equilibrating dried egg powder with the protein solvent.*

TABLE V

EFFECT OF PERIOD EMPLOYED FOR EXTRACTION OF PROTEIN ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Extraction period, hr.			
0.5	1.0	2.0	5.0
215	222	244	253
175	202	208	211
167	176	175	178
78	76	78	84

* *As defined in Table I.*

Suggested Procedure for the Determination of the Refractometric Value

On the basis of the above results, the following procedure was selected as being the most suitable for determining the solubility of egg powder refractometrically. Approximately 4 gm. of egg powder was weighed into a 50 ml. centrifuge tube, mixed thoroughly with 40 ml. of petroleum ether (boiling range 30 to 50° C.), and centrifuged. After decanting the liquid, the procedure was repeated a second time. The powder was spread out on a filter paper to dry at room temperature for about 30 min. One gram of the

defatted powder was accurately weighed into a test-tube (22 × 175 mm.) and 5 ml. of a 5% solution of sodium chloride added. The glass rod of the mixing device was carefully centred vertically in the tube and the tube mechanically shaken at approximately 300 impulses per minute for two hours. A drop of the extract, removed from the test-tube by means of a piece of small diameter, glass tubing, was placed in the Abbé refractometer and the refractive index determined at 25° C.

Interrelation with Other Solubility Tests

It was of interest to compare the refractometric with other known procedures for determining the solubility of egg powders. Determinations of the potassium chloride values, water soluble nitrogen, and crude albumin nitrogen were selected for this purpose. Duplicate determinations were made by each method on 12 samples of egg powder, six of which were products of prime quality, secured from five different Canadian egg driers, while the remaining six were powders secured from two plants and heat-denatured to various extents.

Potassium chloride values were determined on whole powder by a method described previously (6). The part contributed by the fat–protein complex on this determination was also studied by carefully defatting the usual amount of powder employed (2 gm.) prior to extraction with a 10% solution of potassium chloride. The contents of water soluble and crude albumin nitrogen were determined by the tentative standard methods of the A.O.A.C.

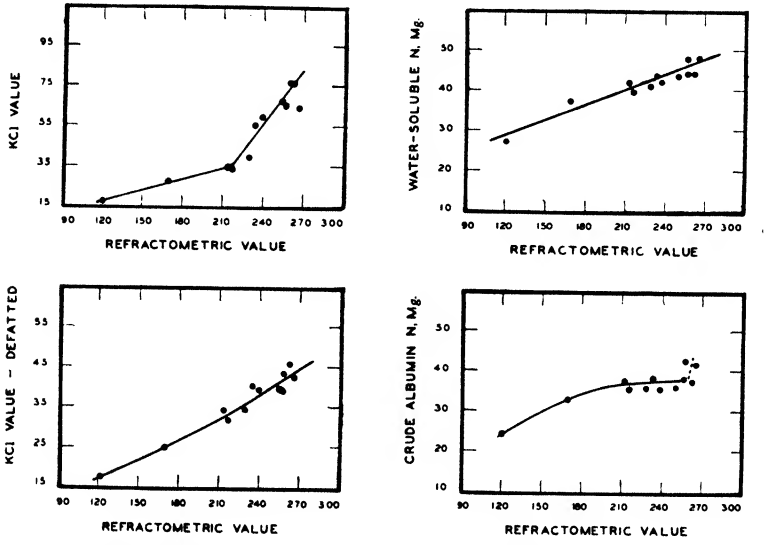


FIG. 2. *The relations between the refractometric value and the potassium chloride values of whole and defatted powder, and the contents of water soluble and crude albumin nitrogen.*

(2). It should be mentioned that some difficulty was encountered in obtaining clear filtrates in the determination of water soluble nitrogen.

The results are shown graphically in Fig. 2. For the rather limited number of samples studied, the refractometric values are for the most part linearly related to the potassium chloride values of defatted powder, and to the content of water soluble nitrogen. A curvilinear relation was obtained for crude albumin nitrogen. The relation between refractometric values and potassium chloride values of whole egg powder is of particular interest. It would appear that potassium chloride values of approximately 35% and higher give a measure of both the amount of soluble protein and of the fat–protein complex present. However, values below about 35% are approximately the same for both whole and defatted powder, indicating that the potassium chloride value in this range is dependent only on the amount of soluble protein present. The fat–protein complex had apparently been thermally decomposed or so altered that the fat could not pass through the filter paper.

Conclusion

The refractometric method described has been successfully used for the determination of the soluble protein content of a large number of samples of egg powders in these laboratories. It was found to be sufficiently precise, rapid, and otherwise suitable for use both in research investigations and routine quality control.

References

1. ALMQUIST, H. J., LORENZ, F. W., and BURMESTER, B. R. Ind. Eng. Chem. Anal. Ed. 4 : 305-306. 1932.
2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 5th ed. A.O.A.C., Washington, D.C. 1940.
3. BAILEY, M. I. Ind. Eng. Chem. Anal. Ed. 7 : 385-386. 1935.
4. LOW TEMPERATURE RESEARCH STATION, CAMBRIDGE, ENG. Private communication.
5. STUART, L. S., GREWE, E., and DICKS, E. E. U.S. Egg Poultry Mag. 48 : 498-503, 524-526. 1942.
6. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
7. URBAIN, W. M., WOOD, I. H., and SIMMONS, R. W. Ind. Eng. Chem. Anal. Ed. 14 : 231-233. 1942.
8. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 194-202. 1943.

DRIED WHOLE EGG POWDER

IV. EFFECT OF MOISTURE CONTENT ON KEEPING QUALITY[1]

By W. Harold White[2] and M. W. Thistle[2]

Abstract

Dried whole egg powders, obtained from three different manufacturers, were adjusted to contain from 2 to 8.5% moisture, and held at temperatures ranging from 7.1° to 43.3° C. Quality was assessed by determination of the fluorescence, potassium chloride, and refractometric values.

Temperature was the most important single factor studied in affecting the keeping quality of dried egg. However, at all temperatures the rate of deterioration increased with increase in the moisture content. To maintain quality during storage and transport, dried egg should contain not more than 5% moisture and preferably 2% or less.

Introduction

Moisture content is usually an important factor in the preservation of dried foods. A low moisture content in dried eggs is normally associated with poor initial quality as a result of too severe drying conditions, unless suitable precautions are taken. At moisture levels of about 8% and higher deterioration due to the growth of moulds has been observed (1). Practical experience has indicated that 5% is a suitable maximum content, but there are few scientific data to confirm this opinion. Consequently it was of importance to investigate the effect of moisture content on quality retention in dried whole egg powder held at various temperatures.

In a recent investigation it was found that the solubility of egg powder, stored at 30° C. and at various relative humidities, decreased less at ultimate moisture contents of about 5% and lower than at the higher levels studied (3). However, this type of experiment suffers from the disadvantage that the moisture content of the powder changes during storage, and in consequence, it is difficult to assess the effect on quality of any particular moisture level. Furthermore, the possibility of a differential effect of moisture content with temperature was not studied.

The present investigation was divided into two parts. In an initial experiment the behaviour of egg powder, adjusted to three moisture levels, was studied during storage at temperatures to which egg powder might normally be exposed. In the main experiment a more extensive series of moisture levels was employed to permit definite conclusions to be made concerning the most suitable moisture content to which egg powder should be dried. These powders were stored at elevated temperatures in order to provide accelerated test conditions.

[1] *Manuscript received May 1, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Material selected from a thesis presented by one of us (W.H.W.) to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as Paper No. 92 of the Canadian Committee on Food Preservation and as N.R.C. No. 1129.

[2] *Biochemist, Food Investigations.*

Materials and Procedure

In the initial experiment egg powder was obtained from a single Canadian producer. Melange consisting of a 1 : 1 mixture of Grades A and B fresh eggs was dried at inlet and outlet temperatures of 108° and 59° C. (227° and 138° F.), respectively. Separate portions of the powder were adjusted to moisture contents of 3.7, 5.2, and 8.5% by aeration with moistened air, and stored at temperatures of 7.1°, 15.6°, 23.8°, and 32.1° C. (45°, 60°, 75°, and 90° F.). Samples were removed for analysis after storage for 1, 2, 4, and 6 months at 7.1° to 23.8° C. and at semimonthly periods during 3.5 months at 32.1° C. Quality of the egg powders was assessed by determination of the fluorescence and potassium chloride values (2, 4). The fluorescence value has been shown to be related to flavour quality (2) whereas the potassium chloride value gives a measure of solubility and the condition of the fat–protein complex present (4, 5).

The above experiment gave information on the behaviour of powders containing various amounts of moisture during storage. However the number of moisture levels studied was insufficient to permit definite conclusions to be made concerning the most suitable moisture content to which egg powder should be dried.

In a second and more extensive experiment, egg powder was obtained from two representative Canadian plants and adjusted to six moisture levels. In Plant I the powder was prepared in a box type drier from a melange consisting of 2 : 1 mixture of storage and frozen eggs at inlet and outlet temperatures of 154° and 65° C., respectively (310° and 150° F.). In Plant II, melange consisting of a 1 : 1 mixture of storage and frozen eggs was dried in a cone type drier at inlet and outlet temperatures of 110° and 50° C., respectively (230° and 130° F.). In each instance the powder was taken directly from the drier and cooled promptly to about 0° C.

The moisture contents of the powders from the two plants were adjusted to approximately 2, 3, 4, 5, 6, and 7%. To achieve this, samples approximately 1100 gm. in weight, contained in large glass dishes, were placed in vacuum desiccators containing either anhydrous calcium chloride or the amount of water required to give the desired moisture content. The desiccators containing water were partially evacuated and closed off. Those containing calcium chloride were evacuated continuously with a Hyvac pump. All powders were thoroughly mixed at intervals of about two days, and samples removed for moisture analysis. After attaining the desired moisture content, the powders were thoroughly mixed and allowed to stand for one week to ensure uniform moisture distribution throughout the sample. These treatments were all made at 4.4° C. (40° F.) to minimize any change in quality.

Sixty-gram samples of the powders of each moisture content from each plant were placed in sealed tin cans, and heated in air ovens at temperatures of 26.8°, 34.9°, and 43.3° C. (80°, 95°, and 110° F.). Samples were removed for analysis after heating for 0.5, 1, 2, 3, 6, and 9 days at 34.9° and 43.3° C. and after 1, 3, 6, and 9 days at 26.8° C. Quality was assessed by measure-

ment of the fluorescence, potassium chloride, and refractometric values. The refractometric value is related to solubility (5). The significance of the other two measurements has been discussed previously.

Results

Initial Experiment

The importance of the various factors studied in affecting the fluorescence and potassium chloride values was assessed by analyses of variance. Since the sampling times employed at 32.1° C. differed from those at the lower temperatures, these data were treated separately. Moisture content and temperature and time of storage all had statistically significant effects on both the fluorescence and potassium chloride values (Table I).

TABLE I

Analyses of variance of the effect of moisture content on the fluorescence and potassium chloride values of dried egg powder stored at 7.1° to 32.1° C.

Storage temperature, ° C.	Source of variance	D.f.	Mean square	
			Fluorescence value	KCl value
7.1°, 15.6°, and 23.8° C.	Moisture content	2	1098**	2032**
	Temperature	2	2700**	3418**
	Time	3	740.1**	1332**
	Stored vs. non-stored	1	650.5**	1001**
	Moisture content × temperature	4	219.3**	5.298
	Moisture content × time	6	44.64**	41.13
	Temperature × time	6	137.9**	302.8*
	Residual	14	11.86	88.04
	Duplicate error	39	0.446	1.479
32.1° C.	Moisture content	2	2511**	844.1**
	Time	6	940.3**	356.4**
	Stored vs. non-stored	1	6998**	6976**
	Residual	14	90.19	24.30
	Duplicate error	24	1.23	0.8229

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

Fluorescence Values

Increases in the moisture content or the temperature or period of storage caused the mean fluorescence values to increase (Table II). The differences were greatest between moisture contents of 5.2 and 8.5%, temperatures of 15.6°, 23.8°, and 32.1° C. and storage periods of two to four months at 7.1° to 23.8° C. At 32.1° C. the mean fluorescence values changed rapidly during the first 1.5 months, and subsequently showed some tendency to decrease. This latter behaviour has been observed previously (6), although

at considerably higher fluorescence values, and was attributed to the decomposition of fluorescent materials.

TABLE II

MEAN VALUES FOR THE EFFECT OF MOISTURE CONTENT ON THE FLUORESCENCE AND POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDER STORED AT 7.1° TO 32.1° C.

Moisture content

Moisture content, %	3.7	5.2	8.5
Mean fluorescence value[1]	28.6	31.2	40.9
Mean fluorescence value[2]	44.0	53.7	68.9
Mean KCl value[1]	66.9	58.7	49.3
Mean KCl value [2]	46.3	38.3	31.8

Temperature

Temperature, °C.	7.1	15.6	23.8	32.1
Mean fluorescence value[1, 2]	26.8	29.9	46.5	55.5
Mean KCl value[1, 2]	66.2	61.9	43.7	38.8

Storage period

Time, months	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	6.0
Mean fluorescence value[1]	23.6	—	27.7	—	30.3	—	—	—	38.4	41.2
Mean fluorescence value[2]	23.6	38.1	50.3	71.6	58.7	74.0	62.7	65.3	—	—
Mean KCl value[1]	70.7	—	69.2	—	57.8	—	—	—	51.8	50.3
Mean KCl value[2]	70.7	50.1	36.9	34.8	31.1	29.1	28.7	28.8	—	—

[1] *Mean values over all other conditions studied for powder stored at 7.1°, 15.6°, and 23.8° C.*
[2] *As for [1], but on powder stored at 32.1° C. only.*

The behaviour of the fluorescence values with variations in temperature or period of storage differed with the moisture content of the egg powder. The fluorescence values, averaged over all storage periods, of dried egg held at 7.1° or 15.6° C. and containing 3.7 or 5.2% moisture did not differ statistically amongst themselves, but were significantly less than that with 8.5% moisture, whereas at the higher storage temperatures the differences with moisture content were all significant. Similarly the fluorescence values of egg powder, averaged over temperatures of 7.1° to 23.8° C., showed relatively slow increase with time at moisture levels of 3.7 and 5.2%, whereas at 8.5% moisture the differences between all storage times were significant. At 32.1° C. the changes in the mean fluorescence values with time were rapid for all moisture contents, with the rate increasing with the moisture content.

Potassium Chloride Values

The mean potassium chloride values for each condition studied decreased with increase in moisture content or temperature or period of storage (Table II). Differences between the three moisture levels were all statistically significant and approximately of the same magnitude. On the average, an increase in temperature from 15.6° to 23.8° C. caused the greatest decrease in potassium chloride values. Differences with time were greatest between one and two months at 7.1° to 23.8° C., and during the first month of storage at 32.1° C. Subsequent changes were small. Thus, as noted previously for the fluorescence values, an increase in moisture content enhanced the rate of deterioration of dried egg powder during storage.

It is obvious from the above results that a storage temperature as low as 7.1° C. is not sufficient to prevent deterioration in dried egg if the moisture content of the powder is high. It would appear that the moisture content should preferably be as low as possible if satisfactory quality is to be maintained under the storage conditions studied.

Main Experiment

Data on the quality of the powders prior to heat treatment are given in Table III. Within experimental error the quality of the samples with various moisture contents from any one plant was the same. However, the overall quality of the egg powder obtained from Plant I was higher than that from Plant II. This difference is presumably due to variations in the predrying and drying practices employed in the two plants. While the moisture levels attained were not integral values they are, for the sake of convenience, referred to as such hereafter.

TABLE III

Initial measurements on dried egg powders secured from two Canadian plants and adjusted to various moisture contents

Adjusted moisture content, %		Fluorescence value		KCl value		Refractometric value	
Plant I	Plant II	Plant I	Plant II	Plant I	Plant II	Plant I	Plant II
2.20	1.97	14.9	17.2	78.2	79.6	269	255
3.00	2.98	15.0	17.3	78.6	77.9	267	251
3.93	3.97	14.8	16.9	76.4	82.1	267	257
4.99	4.90	15.0	16.2	76.9	77.9	267	254
5.76	5.86	14.6	17.1	75.2	76.5	265	254
6.90	6.91	14.6	16.5	75.9	77.9	264	252

Analyses of Variance

The relative importance of the various factors studied in affecting the fluorescence, potassium chloride, and refractometric values was assessed by means of analyses of variance. In making these computations, the data obtained for powders heated at 35.0° and 43.3° C. for 0.5 and 2 days were

disregarded since this information was not available at 26.7° C. The results show that temperature, time of heating, and moisture content usually had the greatest effect on quality, as assessed by the various measurements (Table IV). Variations attributable to differences between plants were of relatively minor importance as compared to the other factors, except for their effect on solubility as determined by the refractometric method.

TABLE IV

ANALYSES OF VARIANCE OF THE EFFECT OF MOISTURE CONTENT ON THE FLUORESCENCE, POTASSIUM CHLORIDE, AND REFRACTOMETRIC VALUES OF DRIED EGG POWDER STORED AT 26.8° TO 43.3° C.

Source of variance	D.f.	Mean square		
		Fluorescence value	KCl value	Refractometric value
Moisture content	5	1772**	1359**	4011**
Temperature	2	14,648**	14,159**	22,020**
Time	3	7715**	4720**	6891**
Plants	1	320.5**	167.1	6786**
Plants × moisture content	5	8.05	11.40	54.16
Plants × time	3	51.27	9.74	131.6
Plants × temperature	2	32.27	124.8	135.5
Moisture content × time	15	217.2**	141.1**	525.8**
Moisture content × temperature	10	383.9**	361.9**	1266**
Time × temperature	6	1471**	2263**	3920**
Residual	91	27.13	43.39	131.4
Duplicate error	144	0.3730	1.333	2.785

** *Indicates 1% level of statistical significance.*

Mean values for each variable, as averaged over all others for the entire experiment, are given for each measurement in Table V.

Regardless of the method of preparation, the keeping quality of dried egg varied directly with its moisture content and the temperature–time treatment to which it was subjected. Differences in quality were least between 2 and 3% moisture and usually greatest between 3, 4, and 5%. The greatest differences with temperature occurred between 34.9° and 43.3° C., which is in agreement with the results of a previous investigation (6). Changes with time in the mean potassium chloride and refractometric values were greatest between three and six days, whereas those in the fluorescence values were of approximately the same magnitude during each of the three-day periods studied. The differences between the mean values of the measurements for the two plants were small, and about the same magnitude as noted initially.

Details of the changes in the fluorescence, potassium chloride, and refractometric values treated in the analyses of variance are illustrated graphically in Figs. 1 to 3, respectively.

TABLE V

THE EFFECT OF MOISTURE CONTENT ON THE MEAN FLUORESCENCE, POTASSIUM CHLORIDE, AND REFRACTOMETRIC VALUES OF DRIED EGG POWDER STORED AT 26.8° TO 43.3° C.

Moisture content

Moisture content[2], %	2	3	4	5	6	7
Mean fluorescence value[1]	21.8	23.7	27.3	31.5	34.4	37.2
Mean KCl value[1]	74.7	71.8	68.7	65.7	63.0	60.7
Mean refractometric value[1]	264	263	257	250	246	241

Temperature

Temperature, ° C.	26.8	34.9	43.3
Mean fluorescence value[1]	18.8	26.5	42.8
Mean KCl value[1]	76.7	71.9	53.7
Mean refractometric value[1]	265	259	236

Storage period

Time, days	1	3	6	9
Mean fluorescence value[1]	17.0	25.5	33.8	40.9
Mean KCl value[1]	76.0	71.8	64.0	57.8
Mean refractometric value[1]	263	261	248	243

Plants

Plant	I	II
Mean fluorescence value[1]	28.2	30.3
Mean KCl value[1]	68.2	66.7
Mean refractometric value[1]	258	248

[1] *As averaged over all other conditions for the whole experiment.*

[2] *Approximate moisture contents, see Table III for actual values.*

Fluorescence Value

An increase in the temperature, time of heating, or moisture content caused an increase in the fluorescence values (Fig. 1). Changes in the fluorescence value with time depended to a considerable extent on the moisture content of the powder and the temperature at which it was heated. At 26.7° C. the relation was essentially linear, the slope of the curve increasing with increase in the moisture content. However, at 35.0° and 43.3° C. there was considerable difference in behaviour between the various moisture levels. At 35.0° C. the fluorescence values of powders containing 5% or more moisture usually increased slowly during the first three days and rapidly subsequently, whereas at 43.3° C. the opposite behaviour occurred. At the lower moisture

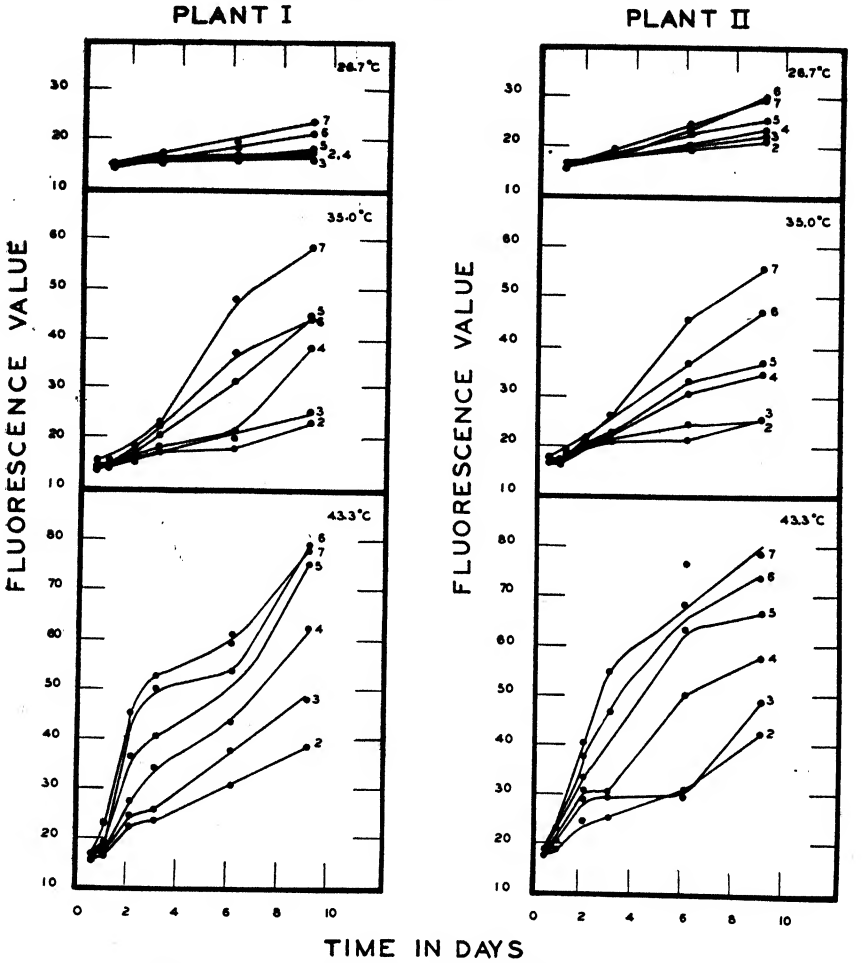


FIG. 1. *Effect of moisture contents of 2 to 7% on the fluorescence values of dried egg powders.*

levels of 2 or 3% the rate of increase of the fluorescence values was usually less after three days at both temperatures.

Variations in the moisture content and the temperature and time of heating had approximately the same effect on the fluorescence values regardless of the plant in which the dried egg was manufactured. This suggests that the differences in quality normally observed between powders from Canadian plants are due primarily to variations in heat treatment and moisture content.

Potassium Chloride Value

The general behaviour of the potassium chloride values with variations in the moisture content, temperature, time of heating, and manufacture of the

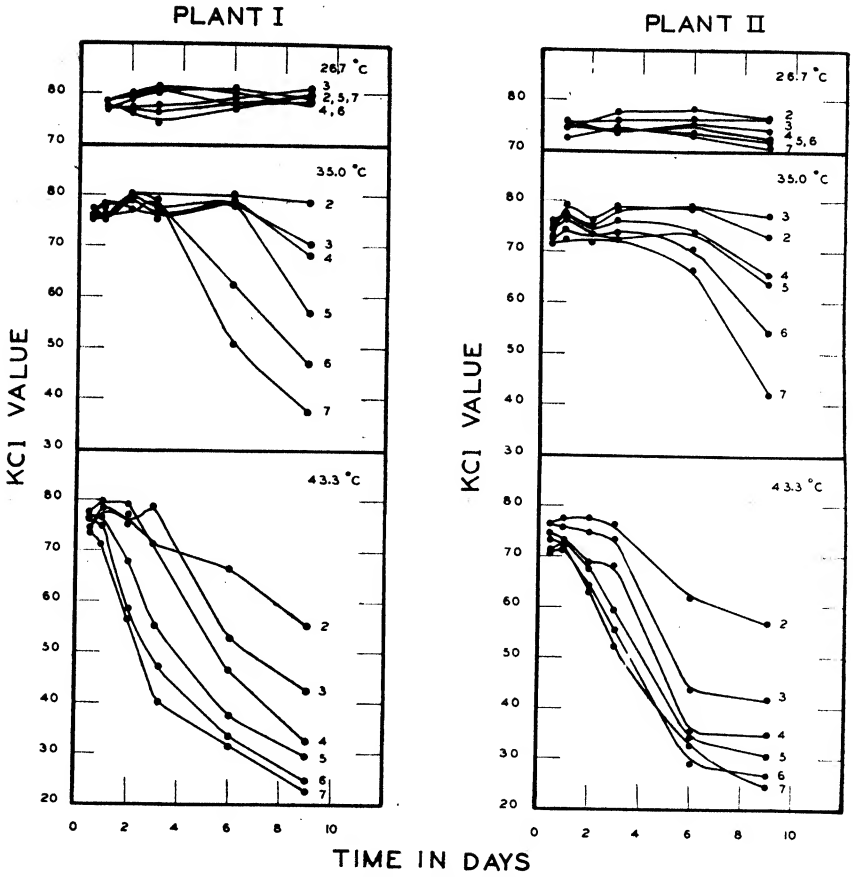


FIG. 2. *Effect of moisture contents of 2 to 7% on the potassium chloride values of dried egg powders.*

powder was usually similar to that noted above for the fluorescence value, except that the trend of the changes was in the opposite direction (Fig. 2). At 26.7° C. the changes in potassium chloride value were usually small and somewhat variable. There was, however, definite indication that the higher moisture contents were associated with lower potassium chloride values. At the higher temperatures the rate of decrease of the potassium chloride values was greater the higher the moisture content and temperature.

Refractometric Values

The refractometric values decreased with increase in the moisture content and temperature and time of heating (Fig. 3). The results obtained at 26.7°C. and 35.0° C. were somewhat variable. Nevertheless, there was a definite

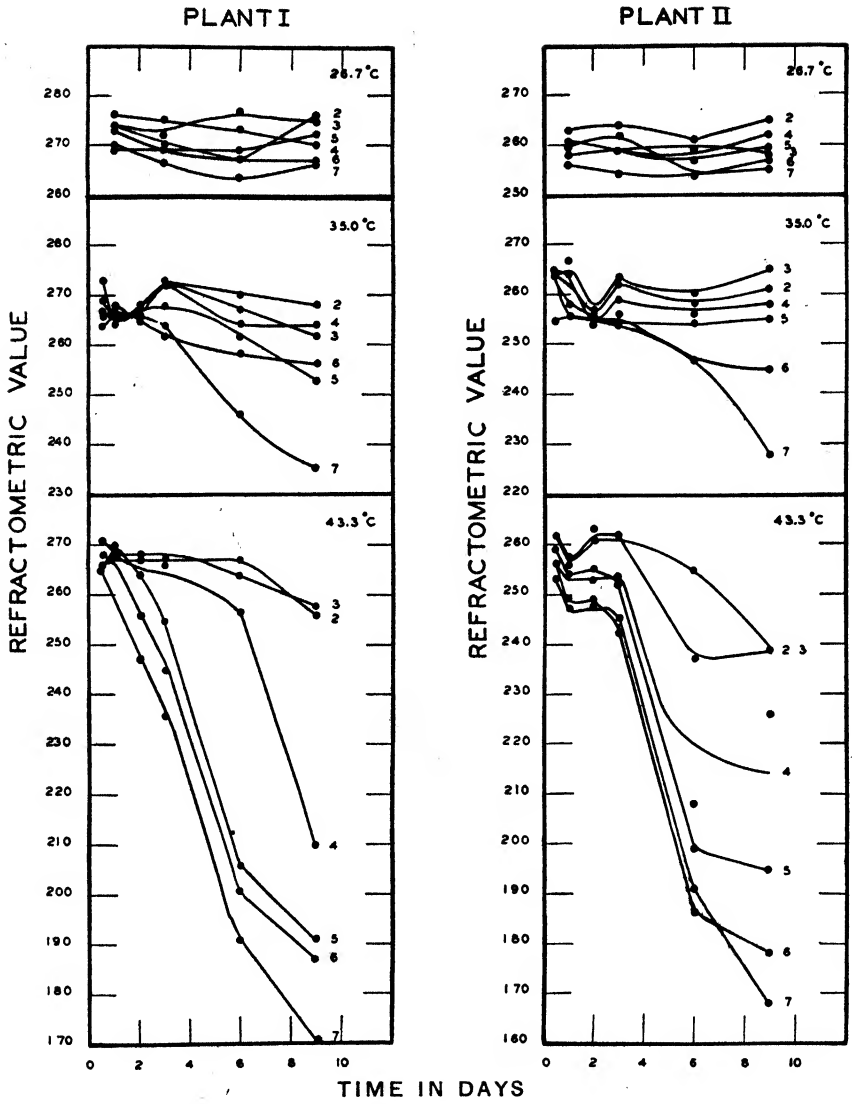


FIG. 3. *Effect of moisture contents of 2 to 7% on the refractometric values of dried egg powders.*

tendency for the refractometric values to decrease more rapidly with time at the higher moisture levels. At 43.3° C. changes in the solubility of powders containing 2 or 3% moisture were relatively slow and similar in character, whereas at the higher moisture levels the solubility usually decreased markedly between three and six days, and more slowly subsequently.

Interrelation of Methods for Assessing Quality

The degree of interrelation of the fluorescence, potassium chloride, and refractometric values was determined by the computation of simple coefficients of correlation. Data were used only for those samples that corresponded to egg powder of quality suitable for export by present Canadian standards, i.e. a fluorescence value of 52 or lower and a potassium chloride value of 40 or higher. It is to be noted that such a selection includes a higher proportion of samples of poor quality than is normally encountered in Canadian dried egg powders. All three methods were significantly interrelated (Table VI). The relative order of the magnitudes of the correlation coefficients is that expected when it is recalled that, on the basis of present knowledge, the fluorescence value is related to overall quality, the potassium value to both overall quality and solubility and the refractometric value to solubility alone.

TABLE VI

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN THE FLUORESCENCE, POTASSIUM CHLORIDE, AND REFRACTOMETRIC VALUES OF DRIED EGG POWDER

Method	Method	
	KCl value	Refractometric value
Fluorescence value	−0.88**	−0.71**
KCl value	—	0.75**

** *Indicates 1% level of statistical significance for the 127 degrees of freedom available.*

Conclusion and Discussion

The major portion of the variations in quality of egg powders studied was attributable to moisture content and the temperature and time of heating. Differences in the constituents of the egg melange and in predrying and drying practices employed in the two plants were of less importance. Since at all temperatures from 7.1° to 43.3° C. deterioration occurred in powder containing as little as 2 to 3% moisture, it appears that there is no limit of moisture content, commercially attainable at present, below which deterioration can be prevented regardless of the temperature to which dried egg is exposed.

The importance of moisture content on keeping quality may be further demonstrated by interpreting the experimental data in terms of the time required for egg powders, of different moisture contents and held at various temperatures, to deteriorate to the lowest quality acceptable for export to England as first grade powders, namely, a fluorescence value of 26. For example, at 7.1° C. such periods for powders containing about 3, 5, and 7% moisture were approximately 3, 3, and 1.5 months, respectively; at 26.8° C., 17, 12, and 6 days; and at 43.3° C., 46, 34, and 26 hours, respectively.

On the basis of this investigation it is now required that the moisture content of all egg powder prepared for export to England shall be as low as

is compatible with the preparation of a powder of satisfactory quality and in no circumstance greater than 5%. While it is obvious that the moisture content should preferably be 2% or lower, the attainment of these levels would probably result in powders of undesirably low initial quality because of the higher drying temperatures usually required by present Canadian drying practice.

Acknowledgments

The authors wish to thank Mrs. Margaret Reid and Messrs. D. A. Fletcher and G. A. Grant for assistance with the analyses, and Mr. W. D. B. Reid for making the statistical computations.

References

1. GIBBONS, N. E. Private communication.
2. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20 : 276-282. 1942.
3. STUART, L. S., HALL, H. H., and DICKS, E. E. U.S. Egg Poultry Mag. 48 : 629-633, 658. 1942.
4. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
5. WHITE, W. H. and GRANT, G. A. Can. J. Research, D, 21 : 203-210. 1943.
6. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 194-202. 1943.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. AUGUST, 1943 NUMBER 8

STUDIES OF WATERFOWL IN BRITISH COLUMBIA

MALLARD[1]

BY J. A. MUNRO[2]

Abstract

Anas platyrhynchos is widely distributed along the Pacific coast. The main winter range extends from coastal Alaska to northern California with the greatest concentration in the lower Fraser Valley of British Columbia and adjacent counties in Washington State. To this coastal plain come summer populations from interior British Columbia, Alberta, Yukon Territory, and Alaska. Here also is a relatively small resident population. A winter population in the interior is increasing in numbers. Migration routes follow the coast and the main river systems, the latter leading to a wide highway of dispersal along the interior plateaux. Band recoveries identify these migration highways and the seasonal movements; they show that mallards follow the same general routes, return in successive years to the same wintering grounds and that population units remain together. On the southern coast nesting commences early and downy young have been seen in March and April. In the interior the majority nest in May and many different types of nesting habitats are occupied. Males leave the females after incubation has started and by early June many, still in full breeding plumage, have gathered in flocks. Subsequently as males start to eclipse they become less gregarious. When the flight feathers are renewed the males again assemble in flocks that later are joined by females and flying young. On the coast one winter population feeds chiefly on seeds and vegetation, secured on flooded fields; another feeds exclusively on salmon eggs and salmon flesh; a third, occupying the littoral, on algae and small marine animals. In the interior the seeds of aquatic plants, more particularly *Scirpus acutus* and *Potamogeton pectinatus* are important foods, so are aquatic insects chiefly Odonata nymphs and corixids. One population in autumn lives almost entirely on grain secured from the fields. The mallard is the duck species of greatest economic importance in British Columbia and the source of a considerable item of revenue to the province. This value is believed to outweigh an economic loss of undetermined proportion brought about by the mallards' consumption of salmon eggs and damage to agricultural crops.

Introduction

The mallard, *Anas platyrhynchos* Linnaeus, is more widely distributed, geographically, in British Columbia than is any other duck species. It is quickly adaptable in respect of nest and food requirements and both its breeding and winter ranges include a variety of diverse habitats. It is abundant and in certain localities during late autumn may outnumber the total of all other ducks present. It is the species bearing the heaviest hunting

[1] *Manuscript received April 28, 1943.*

Contribution from the National Parks Bureau, Department of Mines and Resources, Ottawa, Canada.

[2] *Chief Federal Migratory Bird Officer, British Columbia.*

pressure and yet is the only one that appears to have maintained its numbers or actually increased during the past 20 years.

The present paper records the distribution and migration of the species in western North America and the behaviour, general life history, numerical status, and food habits as studied in British Columbia. The section on distribution and migration is based on an analysis of data provided by the recovery of banded mallards, and supplemented by field observations conducted since 1911. The section on food habits is the sum of field observations during this period supplemented by the study of food in the stomachs of 218 specimens.

Distribution and Seasonal Movements

Coast Region

During the years 1928 to 1940, inclusive, a total of 17,395 mallards was banded at five stations in British Columbia. The ducks were captured in poultry netting traps baited with wheat and constructed so that a funnel-shaped entrance was in shallow water and the greater part of the trap on land. A total of 3387 bands has been recovered and reported as at January 31, 1943. Of this total 2516 were taken in the first year after banding and the remainder as follows: 2nd year, 510; 3rd year, 189; 4th year, 186; 5th year, 35; 6th year, 24; 7th year, 14; 8th year, 2; 9th year, 1; 10th year, 1.

Five banding stations were operated as follows: (1) McGillivray Creek Game Reserve, near Sardis, conducted as a joint project of the National Parks Bureau and the Provincial Game Commission; (2) Pitt Meadows near Port Coquitlam, operated by the Provincial Game Commission on the property of Mr. A. L. Hagar; (3) Westham Island, at the mouth of the Fraser River, operated by Mr. George C. Reifel; (4) Vaseaux Lake, Okanagan Valley; (5) Buffalo Lake, Cariboo District, operated by the National Parks Bureau.

The distance from Station 1 to Station 3 is approximately 55 miles true west, the distance from Station 3 to Station 2 approximately 22 miles true northeast, and the distance from Station 1 to Station 2 approximately 30 miles true northwest. Being situated close together on the wintering ground along the Coastal Plain, Stations 1, 2, and 3 are regarded as a unit and the data have been combined for statistical purposes. Stations 4 and 5, situated on migration flyways in the interior and 180 airline miles apart, are treated separately. The totals of mallards banded and the totals of returns for each station are shown in Table III. A summary of returns by provinces, states, and territories is given in Table IV. See also Fig. 1.

Recoveries are classified in the following categories, viz., Coastal Plain, current year, 1687 or 49.9%, later years 1014 or 29.5%, Table V; southeast and west Coastal Plain, current, 48 or 1.5%, later years, 184 or 5.7%, Table VI; winter recoveries north of Coastal Plain, 9 or 0.2%; transient spring and summer, first year, 43 or 1.2%, later years, 33 or 1%; transient autumn, north of Coastal Plain, 225 or 6.8%, Table VIII; Vaseaux Lake, 68 or 2%, Table X; Buffalo Lake, 76 or 2.2%, Table XI.

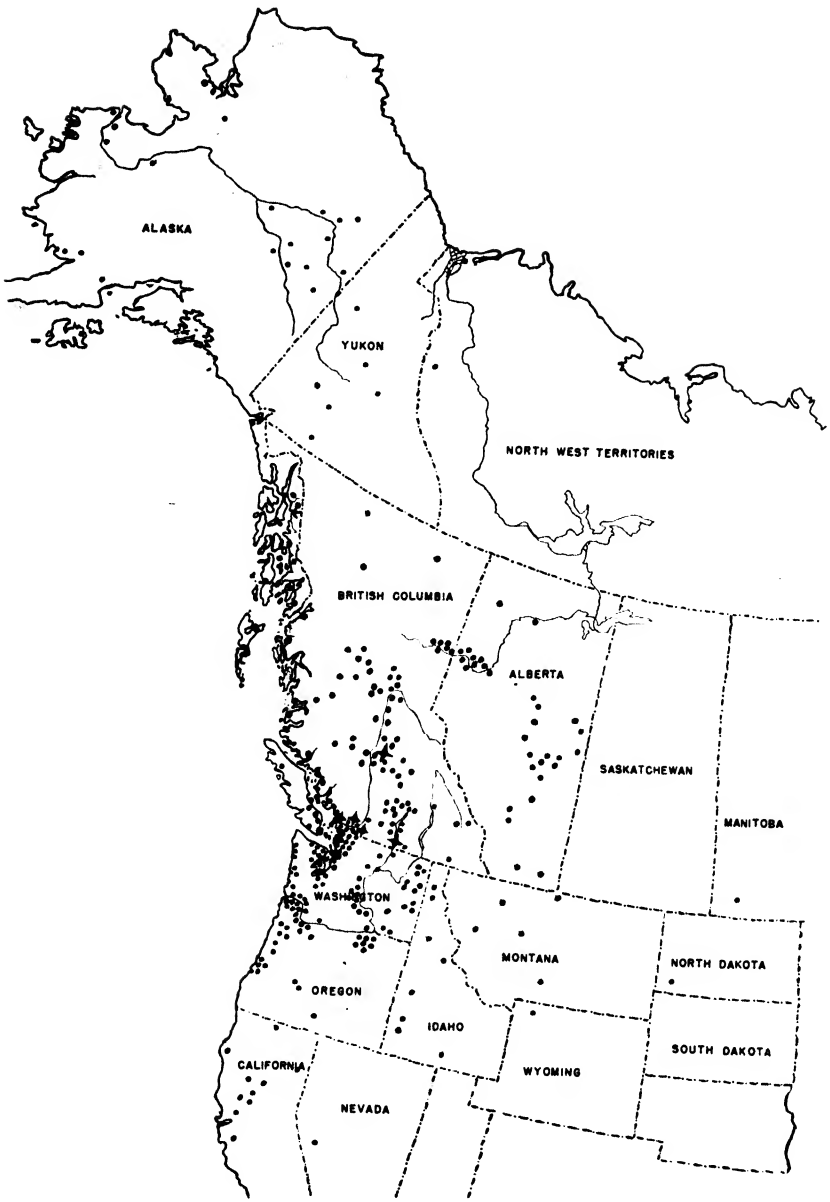


FIG. 1. *Dispersal of mallards banded in British Columbia. Each dot represents one or more recoveries. One recovery from Missouri and one from Illinois are not included; each star represents a banding station.*

Winter

The wintering ground of mallards on the Pacific Coast is included within a wide latitudinal range from Alaska to California (Table VI). Current recoveries, that is, bands recovered during the autumn and winter banding season, and recoveries in later years bring out the fact that the greatest concentrations take place in a rather limited area referred to here as the Coastal Plain. The Coastal Plain is defined as the delta and valley of the Fraser River and its tributaries east to the vicinity of Rosedale, the southeast coast of Vancouver Island and the Gulf Islands, and that part of the Puget Trough in the Washington counties of Whatcom, Skagit, Snohomish, King, San Juan, and Island. This unit of territory probably constitutes the most densely populated area in western North America. A total of 2701 band recoveries come from this region as compared with a total of 241 from all other points on the coast.

TABLE I

FOOD OF MALLARD, TOTAL PERCENTAGE VOLUME

Locality and number of specimens		Salmon eggs	Corixids	Odonata	Miscellaneous insects	Molluscs	Miscellaneous animals	Round-stem bulrush seeds	Miscellaneous seeds	Grain	Miscellaneous vegetation
Lower Fraser Valley	90								62.61	2.94	34.65
Pitt Meadows	6								100.00		
Boundary Bay	3					35.00	45.00		20.00		
Southern Vancouver Island	5				33.60	4.00			20.40	18.00	24.00
Departure Bay	2					7.50	40.00		5.00		47.50
Seal Island	1					50.00					50.00
Henderson Lake	6	65.00					35.00				
Quinsome Lake	5	74.00					10.00				16.00
Swan Lake, Okanagan	85		18.45	22.94	0.56	0.83	0.07	28.54	9.24	6.78	12.59
Okanagan Region	5		1.00	2.25			13.25		57.00		26.50
Cariboo Region	9		0.11	1.11	2.44	13.00	11.00	13.89	25.42		33.00
Babine Lake	1	95.00			5.00						

The main flights reach the Coastal Plain usually in November, the time varying considerably from year to year and apparently being largely controlled by temperature; if freezing conditions occur in the north early in the autumn there is an early migration while high temperatures in the north retard the migration (Figs. 2, 3). The following show the dates in each banding year when the largest number were captured at Station 1 and probably approximate the time of the migration peak for these years:

Date	No. captured	Date	No. captured
1928, November 9	190	1935, November 12	127
1930, November 4	84	1936, October 25	42
1931, November 14	87	1937, November 2	209
1932, November 22	130	1938, December 2	113
1933, December 10	180	1939, November 16	184
1934, November 6	245		

A large number of the 1687 current recoveries on the Coastal Plain were made shortly after the ducks were banded, some actually on the same day and within a few miles of the banding station, others several months later either at places close to the station or at relatively distant points within the Coastal Plain. Thus 639 of the 1614 recoveries from mallards banded at

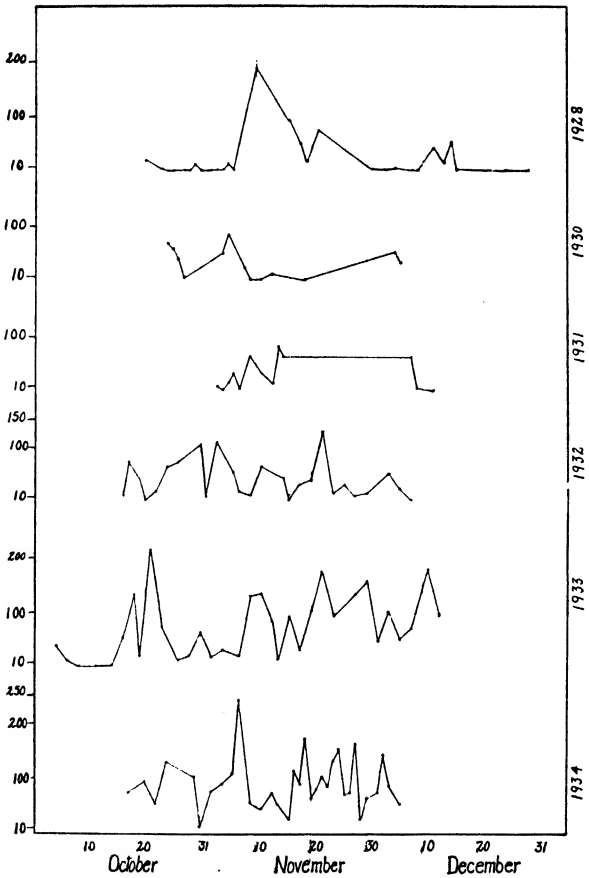


FIG. 2. *Daily catch of mallards at Station 1, October to December inclusive; 1928 and 1929 to 1934.*

Station 1 came from places one to six miles distant, 17 came from Vancouver Island, 558 from points between these extremes of east and west, and 400 from Washington State. Two of the latter were short-time recoveries indicating the distance travelled in one day. One banded at Station 1 on December 22, 1933, was shot at Mt. Vernon, Wash., approximately 45 miles south, on the same day, and one banded at Station 1, November 17, 1928, was shot at Blaine, Wash., 25 miles southwest on the following day.

Results at Station 3 were similar. Fifty were recovered at points one-half to five miles from the station, one at Sardis, 13 from intermediate points, and nine from Washington State.

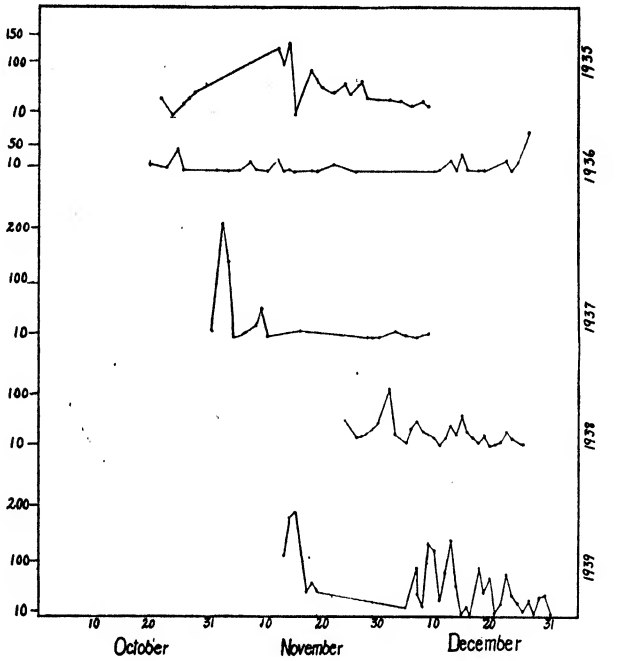


FIG. 3. *Daily catch of mallards at Station 1, October to December inclusive; 1935 to 1940.*

In addition to the recoveries of bands from birds shot, there are many records of banded birds subsequently retrapped and released. Some individuals entered the trap on 15 or more consecutive days, others on as many as 20 days during a six week period. Not all such occurrences were recorded at the time but for Station 1 a partial record is available covering the banding periods from 1930 to 1940 inclusive. During this time there were 141 recorded recoveries in which the time elapsing between banding and last subsequent recovery approximated one week; 123, two weeks; 65, one month; 26, two months; 13, three months; and 3, four months. These data, which have not been tabulated, are of similar value to those referred to as current recoveries.

The significance of current trap recoveries alone could be questioned because the attraction of food in the trap may have been the factor controlling the return of the birds. But as these recoveries parallel local recoveries of birds shot they are considered acceptable. It is necessary also to mention another factor of considerable importance that influenced the movements of mallards in the region adjacent to Station 1. This station was located on a Game

Reserve where shooting was prohibited. The protection thus afforded and the presence of grain were responsible for a daily concentration of mallards in this area.

In spite of these two potent attractions, however, there was considerable variation in the movements of individual mallards. Some returned daily, others after an absence of a week, a month, or longer. Thus there seems no reason to modify the conclusions summarized in the following paragraph.

In mild, wet winters on the Coastal Plain fields are flooded, thus ensuring a plentiful food supply for mallards. It is well known through observation that flocks are to be found daily on certain feeding grounds in various localities. There was no means of knowing, however, whether these populations consisted of the same individuals present from day to day, or week to week, or whether one population moved out and was replaced by another. Data provided by banded birds shot and by current trap recoveries show that some individuals are sedentary while others move from one district to another and back again. There is no general shift of the population except that brought about by a sudden drop in temperature which freezes surface water and drastically reduces the food supply.

The most definite fact that emerges from the study of current recoveries is that mallards concentrate on the Coastal Plain and that this area probably supports the largest winter population on the Pacific Coast. This conclusion is strengthened by the high percentage of later recoveries from the region. These, 1014 in number, show that mallards return to the same wintering grounds on the Coastal Plain and trap recoveries show that some return to the precise locality. Thus a mallard banded at Station 1 on November 19, 1933, was recaptured there on November 26, 1934; another banded at Station 1, December 19, 1938, was recaptured there December 22, 1939. There are 78 records of banded mallards that were recaptured a year or more later at the same station (Table XIII).

These data analysed below further emphasize the point. Station 1. Eight hundred and sixty-two recoveries of which 187 or 21.7% came from localities within 15 miles of the station; first year, 86; second year, 70; third year, 15; fourth year, 10; 2 each in fifth, sixth, and eighth years. Station 2. One hundred and ten recoveries of which 45 or 40.5% came from localities within 15 miles of the station; first year, 27; second year, 15; 1 each in fourth, fifth, and sixth years. Station 3. Forty-two recoveries of which 18 or 42.8% came from localities within 10 miles of the station; first year, 11; second year, 5; 1 each in fifth and tenth years.

Furthermore there is a relatively large amount of evidence suggesting that units of population remain together on the wintering ground in successive years and, presumably, visit the same localities to nest in summer. A total of 32 records has been made in which a male and female, or two males, or two females banded on the same day at the same place were recovered a year or more later, some couples on the same day at the same place, others in the

TABLE II

FREQUENCY OCCURRENCE OF IDENTIFIED FOOD ITEMS IN 218 MALLARD STOMACHS

Sockeye salmon, *Oncorhynchus nerka*	Eggs	10
Salmon, *Oncorhynchus* sp.	Bones, flesh	2
Unidentified fish (Pisces)	Bones	1
Leech, Hirudinea	Egg cases	1
Amphipod, unidentified marine form		1
Amphipod, *Hyalella azteca*		2
Shore crab, *Hemigrapsus* sp.		1
Dragonflies (Odonata)	Nymph	45
Damsel flies (Odonata)	Nymph	9
Water boatmen, Corixidae		53
Caddis, Trichoptera	Larva	3
Micro-trichoptera	Larva	2
Beetle (Coleoptera), unidentified terrestrial form		4
Beetle (Gyrinidae)		5
Beetle, unidentified aquatic form	Larva	1
Beetle, *Dytiscus* sp.	Larva	1
Crane fly (Tipulidae)	Larva	1
Fly (Diptera)		1
Midge (Chironomidae)	Larva	5
Cricket (Orthoptera)		1
Snails, unidentified marine forms		2
Snails, *Littorina scutulata*		2
Snails, unidentified freshwater forms		18
Snails, *Planorbis* sp.		1
Snails, *Limnea* sp.		1
Clam, *Pisidium* sp.		1
Bryozoa	Statoblasts	3
Marine algae		1
Muskgrass, *Chara* sp.	Branches	1
Muskgrass, *Chara* sp.	Oospores	4
Filamentous algae, *Spirogyra* sp.		1
Horsetail, *Equisetum* sp.	Roots	1
Bur-reed, *Sparganium* sp.	Seeds	46
Cat-tail, *Typha latifolia*	Seeds	1
Pondweeds, *Potamogeton heterophyllus*	Seeds	7
Potamogeton pusillus	Seeds	9
Potamogeton foliosus	Seeds	27
Potamogeton foliosus	Leaves	3
Potamogeton pectinatus	Leaves	4
Potamogeton pectinatus	Seeds	71
Ditch grass, *Ruppia occidentalis*	Seeds	2
Grass (Gramineae)	Leaves	24
Barnyard grass, *Echinochloa* sp.	Seeds	2
Wild oat, *Avena fatua*	Seeds	41
Cultivated oat, *Avena sativa*	Seeds	3
Wheat, *Triticum vulgare*	Seeds	19
Sedge, *Carex* sp.	Seeds	12
Carex exsiccata	Seeds	4
Carex vesicaria	Seeds	1
Bulrush, *Scirpus acutus*	Seeds	106
Scirpus americanus	Seeds	1
Spike rush, *Eleocharis palustris*	Seeds	14
Eleocharis obtusa	Seeds	1
Dock, *Rumex maritimus*	Fruits	1
Bindweed, *Polygonum convolvulus*	Seeds	20
Knotweed, *Polygonum aviculare*	Seeds	3
Smartweed, *Polygonum amphibium* or *Muhlenbergii*	Seeds	18
Polygonum lapathafolium	Seeds	31
Polygonum hydropiper	Seeds	3

TABLE II—*Concluded*

FREQUENCY OCCURRENCE OF IDENTIFIED FOOD ITEMS IN 218 MALLARD STOMACHS—*Concluded*

Smartweed, *Polygonum acre*	Seeds	2
Polygonum persicaria	Seeds	5
Polygonum hydropiperoides	Seeds	10
Goosefoot, *Chenopodium* sp.	Seeds	4
Orach, *Atriplex* sp.	Seeds	2
Pond lily, *Nuphar polysephalum*	Seeds	1
Penny cress, *Thlaspi arvense*	Seeds	4
Ball mustard, *Nestia paniculata*	Seeds	3
Rose, *Rosa* sp.	Seeds	1
Water milfoil, *Myriophyllum spicatum*	Seeds	9
Myriophyllum spicatum	Leaves	1
Hornwort, *Ceratophyllum demersum*	Leaves	2
Ceratophyllum demersum	Seeds	3
Dogwood, *Cornus occidentalis*	Seeds	1
Buckbean, *Menyanthes trifoliata*	Seeds	2
Mint, *Mentha* sp.	Seeds	1
Bedstraw, *Galium* sp.	Seeds	2

same general locality on approximately the same dates (Table XIV). When the chances operating against such dual recoveries are considered, the number of such cases is impressive. Therefore the conclusion that population units winter and migrate together and probably nest in the same locality, is inescapable.

Two recoveries of juvenals banded in the interior of British Columbia and recovered the same year at localities near the mouth of the Fraser River are of sufficient interest for inclusion here. The particulars are: juvenal, banded at Kleena Kleen, July 18, 1933, recovered at Douglas Island, October 28, 1933; juvenal, banded at Swan Lake, Peace River, June 24, 1941, recovered at Sea Island, December 6, 1941.

South of the Coastal Plain the concentration of mallards is less pronounced. In migration a wide dispersal from the eastern counties of Washington and Oregon to the outer coasts of these states is general (Table VI). The most important wintering ground, as indicated by banding recoveries, is an area adjacent to the Columbia River mouth. Sixteen current and 42 later recoveries represent this area. The remainder of the total 174 recoveries are from other widely separated localities.

Details of 18 recoveries are given in Table VII and several of these give some evidence of the time taken in travelling between the banding station and the place of recovery. One bird banded on November 2, 1937, was killed five days later at Klamath Falls, Oregon; another banded November 6, 1932, was killed in Multnomah County, Oregon, three days later. Detracting from the value of these records unfortunately is the fact that it cannot be known how long the bird had been on the scene of its recovery before

being shot. The conclusion reached through a study of these data is that a small but constant stream of migration flows south and east from the Coastal Plain. They suggest that this migration represents one or several populations distinct from those wintering in areas farther north.

There are a few recoveries that appear to be examples of a wandering, vagrant habit exhibited by a small percentage of the population. The particulars are:

Banding station	Date banded	Locality where recovered	Date recovered	Distance and direction travelled
1	Nov. 7, 1933	Squamish, B.C.	Nov. 8, 1933	70 miles N.W.
1	Nov. 9, 1928	Liberty, Mo.	Dec. 18, 1928	1500 miles S.E.
1	Nov. 17, 1934	Mara, B.C.	Dec. 30, 1934	170 miles N.E.
1	Nov. 17, 1934	Yakima Co., Wash.	Dec. 16, 1934	185 miles S.E.
3	Oct. 3, 1932	Yakima Co., Wash.	Dec. 4, 1932	195 miles S.E.
3	Nov. 27, 1932	Great Falls, Mo.	Dec. 23, 1932	550 miles S.E.
Portland, Oregon	Sept. 24, 1928	Mission, B.C.	Oct. 15, 1928	260 miles N.

The extent of distribution north of the Coastal Plain is indicated by the following recoveries made in the second year after banding and in later years: Malaspina Inlet, January, 1935; Toba Inlet, December 27, 1941; Loughboro Inlet, December 16, 1934; Knight Inlet, January 12, 1936, February 10, 1934; Bond Sound, December 11, 1935; Moresby Island, November 28, 1935; Masset Inlet, December 23, 1934; Craig, Alaska, January 23, 1937. It may be said that field observations show that a much larger winter population exists north of the Coastal Plain than the small number of these recoveries would indicate.

Spring Migration

A migration northward along the coast and northeast into the interior of British Columbia, Alberta, Alaska, and Yukon Territory commences in late February, is at its height in March and continues through April. At this time flocks of transients are conspicuous on small lakes and sheltered bays of the sea along the Coastal Plain. The size of these gatherings is indicated by the following counts: Quamichan Lake, March 10, 1934—100; Boundary Bay, March 15, 1934—300±; Agassiz, March 29, 1935—250±; McGillivray Creek Game Reserve, March 30, 1935—300±; March 31, 1935—500±.

The only information available concerning points farther north refers to Port Hardy, north end of Vancouver Island, where a migratory movement has been observed between April 20 and May 10 (Allan Lyon, personal letter) and to McClinton Creek, Queen Charlotte Islands, where migrating flocks, numbering up to 65, were recorded during the last two weeks of April, 1935.

Summer

Following the spring departure of wintering flocks from the coast the residual population is relatively small and scattered. In British Columbia

the mallard nests from southern Vancouver Island to the Queen Charlotte Islands and from the Canada–United States boundary to Alaska. It is resident on the coast region in the sense that mallards are present at all times of the year. To what extent local nesting populations are sedentary and to

TABLE III

NUMBER OF MALLARDS BANDED IN BRITISH COLUMBIA AND TOTAL RECOVERIES

Banding periods	Number banded			Total recoveries	Recovery, %
	♂	♀	Totals		
Station No. 1—					
1928, Oct. 20 – Dec. 31			643	178	27.7
1930, Oct. 21 – Dec. 5	176	290	466	124	26.6
1931, Nov. 3 – Dec. 8	240	204	444	67	15.0
1932, Oct. 16 – Dec. 9	500	585	1085	292	26.9
1933, Mar. 29 – April 18	41	55	96		
1933, Oct. 4 – Dec. 12	923	1489	2412	615	24.5
1934, Jan. 28 – Mar. 14	259	268	527		
1934, Oct. 7 – Dec. 5	1280	1687	2967	674	19.2
1935, Mar. 5 – April 11	4	5	9		
1935, Oct. 22 – Dec. 31	572	581	1153	251	21.6
1936, Jan. 18 – April 11	65	83	148		
1936, Oct. 10 – Dec. 28	195	197	392	103	19.0
1937, Mar. 19 – April 4	75	79	154		
1937, Oct. 31 – Dec. 12	251	287	538	126	18.2
1938, Nov. 24 – Dec. 31	385	431	816	130	15.9
1939, Jan. 1 – April 19	209	302	511		
1939, Nov. 14 – Dec. 31	1066	902	1969	333	13.4
1940, Jan. 1 – Mar. 31	140	133	273	34	12.4
			14,602		
Station No. 2—					
1933, Feb. – April			310	52	16.7
1934, Feb. – April			570	84	14.7
1935, Feb. – April			438	27	6.1
			1318		
Station No. 3—					
1932, Oct. 3 – Dec. 31			241	47	19.5
1933, Jan. 1 – Feb. 19			83		
1933, Sept. 4 – Dec. 17			501	102	17.4
1934, Oct. 9 – Dec. 31			30	2	6.6
1935, Jan. 1 – Jan. 20			15	2	13.3
			869		
Station No. 4—					
1931, Nov.			48	7	14.6
1932, Nov. 1 – Nov. 20	73	62	135	35	25.9
1933, Oct. 30 – Nov. 9	47	15	62	26	41.9
			245		
Station No. 5—					
1932, Aug. 14 – Oct. 6			143	35	24.4
1933, Aug. 30 – Oct. 20	110	108	218	41	18.8
			361		
TOTALS	6611	7763	17,395	3387	
				Average, %	19.4

what extent they are replaced in winter by transients from elsewhere is not known.

Concerning southern Vancouver Island there is some evidence in the form of banding data that suggests the existence of a strictly resident and sedentary population. This evidence is that all the 13 recoveries from a minor banding project at Elk Lake, during the period August to January in several years, were made within 30 miles of the place where banded. Of these 10 were recovered within a week to four months after banding; one was recovered after 11 months and two in the fifth year after banding.

A definite conclusion regarding the status of summer populations in the southern coast region is complicated by the following factor. Since the early settlement of the region many farmers have practised the domestication of wild mallards and for a long period also hunters propagated them for use as live decoys; so also the so-called call ducks and other strains of domestic mallards were crossed in captivity with wild stock. Through the years a great many birds of mixed strain were liberated and they seem to have bred with, and gradually been absorbed by, the wild stock. Certain populations of mixed stock established and resident on protected waters near urban centres constantly were being replenished by wild mallards and at the present time all have the characteristic appearance of the wild birds. They are tamed to fearlessness by frequent feeding but are not confined and make flights to and from the sea. In winter the flocks are joined by baldpate, canvas-back, and other ducks.

It seems likely that the domestication and subsequent liberation of wild mallard, the infusion of domestic strains, and the feeding of large numbers on protected areas and, formerly, on duck-hunting clubs has increased the local nesting population and to some extent at least has modified their seasonal movements. Nevertheless it can be asserted that the numbers of the resident coast population are relatively small, certainly not large enough to materially affect the conclusions summarized above concerning the movements of the large transient and winter populations.

Interior Region

Spring Migration

Spring observations in the Okanagan Valley over a period of years show that in early March flocks of wintering mallards break into smaller units, some to travel north, others to local nesting grounds. Somewhat later appear birds that have wintered along the coast; for the most part these come from points south of the Coastal Plain.

The interior plateaux of the Nicola, Kamloops, and Cariboo regions form a wide highway of migration and dispersal to the Peace River districts of British Columbia and Alberta, to the Chilcotin, and to nesting grounds in central and northern British Columbia, Alaska, and the Yukon. On this highway mingle flocks that have travelled from the coast by way of the Columbia–Okanagan as noted above, by the Fraser–Thompson, and by the

Harrison-Lillooet and Seton Lake systems. The 43 first spring recoveries of mallards banded on the Coastal Plain, Tables VIII and IX, indicate, with some degree of accuracy in respect of time, the northward progress of this migration. The following records are arranged from south to north: Lac La Hache, B.C., April 30, 1934; Horsefly, B.C., April 6, 1934; Woodpecker, B.C., April 21, 1934; Stuart Lake, B.C., April 26, 1934; Crooked River, B.C., April 14, 1934; Fort Fraser, B.C., April 15, 1934; Dawson Creek, B.C., April 30, 1936; Athabasca, Alta., May 9, 1934; Fort Vermilion, Alta., May 14, 1934; White Horse, Yukon, May 14, 1934; Galena, Alaska, May 21, 1934. The 33 later year recoveries, Table VIII, serve to confirm the above definition of the migration routes.

Some information on the spring migration is available from other sources. The earliest record for the species at Chezzacut Lake in the Chilcotin is March 22, 1941—2; it became common on April 1, 1941—100± (F. M. Shillaker, personal letter). At Francois Lake 1500± were counted on April 16, 1941 (J. Sugden, personal letter). In 1938 it was first seen at Tetana Lake on April 14 and became abundant on April 21 (6). The earliest record for Atlin is April 14, 1938 (7).

Autumn Migration

Usually by the end of September a general dispersal, influenced in some sections by the opening of the hunting season, has taken place. In the Cariboo region, where the extent of the feeding grounds is large, part of the population shifts to more remote feeding grounds but observations show that a definite southern movement is also general at this time. In the Okanagan Valley, where feeding grounds are of much less extent, a general exodus takes place following the opening of the hunting season. In the course of the ensuing few weeks a gradual influx of transients again builds up the population.

The following counts at Swan Lake, Okanagan, commencing 10 days after the opening of the hunting season in 1942, illustrate this point:

Sept. 29	Oct. 7	Oct. 9	Oct. 13	Oct. 19	Oct. 30	Nov. 5
40	30	25	60	400	700±	1000±

Important data concerning the autumn migration were provided by the 76 recoveries from 361 mallards banded at Buffalo Lake. Evidence that individual mallards use the same migration route in successive years is afforded by four recoveries of birds banded in the autumn and recovered at or near the same place in later autumns. The particulars are:

Date banded	Locality where recovered	Date recovered
September 21, 1932	Lac La Hache	September 20, 1935
September 21, 1932	Buffalo Lake	October 13, 1937
September 21, 1932	Buffalo Lake	October 19, 1933
October 6, 1933	Buffalo Lake	September 28, 1934

The 55 first year autumn recoveries and the 21 recoveries in later years from mallards banded at this station, Table XI, show clearly that two migration routes are followed, one by the Columbia–Okanagan to wintering grounds chiefly along the Columbia River, the other by the Fraser–Thompson to the Coastal Plain (Table XII).

The 68 recoveries from 245 mallards banded at Vaseaux Lake in autumn are in two categories, the first derived from a population that apparently is resident in the Lower Okanagan Valley and discussed later, the second from a transient population (Table X).

Further evidence that mallards use the same migration route in successive years was afforded here by subsequent trap recoveries of three mallards banded at this station, the details being: one banded November 9, 1932, was recovered November 8, 1933; two banded November 10, 1932, were recovered November 7, 1933.

No first year recoveries, and only two in later years, were made on the Coastal Plain from mallards banded at this station, while nine were taken in the first autumn and winter at points to the south and east. The evidence is clear that the main route to the wintering ground is along the Columbia–Okanagan system. The particulars are given below.

Date banded	Locality where recovered	Date recovered
November 7, 1932	Spokane Co., Wash.	December 5, 1932
8, 1932	Okanogan Co., Wash.	Winter 1932
15, 1931	Chelan Co., Wash.	December 6, 1931
10, 1932	Yakima Co., Wash.	November 23, 1932
16, 1931	Walla Walla Co., Wash.	December 11, 1931
10, 1932	Whitman Co., Wash.	December 8, 1932
12, 1932	Polk Co., Oregon	November 24, 1932
12, 1932	Columbia Co., Oregon	December 14, 1932
12, 1932	Nez Perce Co., Idaho	Autumn 1932

The portion of British Columbia east of longitude 118° W., including the districts of east and west Kootenay, is represented by only five recoveries of banded mallards, one being taken in the first autumn after banding, the others in autumns of later years. They were recovered at widely separated points, viz., Revelstoke, Donald, Kootenay Lake, Grand Forks, and Radium Hot Springs. Mallards are fairly plentiful in the region and it might be expected that a large number originating here would have been banded on the Coastal Plain had this been their wintering ground or on the route of their migration. As such was not the case it may be inferred that the five recoveries representing this region are examples of individuals wandering from the normal course of migration. Possibly the mallards of the region are more closely associated with a population that nests in Alberta and migrates by a route east of the Rocky Mountains to a wintering ground on the Gulf of Mexico. This theory is strengthened by the fact that five mallards

banded at Moiese, Mont., were recovered in the region, two at Wynndel and one each at Sirdar, Kootenay Flats, and Golden (Table XV). Moiese is 170 airline miles southeast of Wynndel.

Winter

The presence of a winter population in the Lower Okanagan Valley has been mentioned. In a total of 68 recoveries from mallards banded at Vaseaux Lake 45, or 66.3%, were made locally, 18 in the current year and 27 in later years, 12 being taken in December when the normal migration season has ceased.

A winter population in the northern part of the Okanagan Valley has increased in size very considerably within the past decade during which time mallards have taken to feeding on the grain fields. This is discussed in the section dealing with food habits. In the winter of 1911, and in subsequent winters for approximately 10 years later, the majority left in late autumn when the small lakes and ponds froze over and only a small population, fluctuating in numbers from year to year, remained. These frequented open stretches of streams or gathered on the shore of Okanagan Lake where they fed upon vegetation that drifted on to the frozen shore. In the Okanagan Landing region during the winter of 1917–18 only eight were observed. No other precise counts for this period are available. In the winter of 1921–22 a flock estimated at 100 wintered on Shuswap Lake near Sicamous. This was a severe winter and although the birds were fed by local residents many of them died.

By 1931 it was observed in the Vernon district that the winter population was increasing in numbers as the following counts testify, viz.: Okanagan Landing, December 1, 1931, flocks totalling 100, December 30, 1932, 60; Coldstream, near Vernon, January 1, 1933, 150±; Okanagan Lake, north arm, December 19, 1935, 350±. The following five winters were exceptionally mild and it seems probable that the numbers of the wintering population increased but no definite information is available except for the winter of 1939–40. In this season large flocks estimated to total 2000 remained in the Kelowna and Vernon districts and a winter population also was reported from the Kamloops district.

Small winter populations are reported from Nelson in the west Kootenay and from Cranbrook in the east Kootenay. The most northerly interior record is for the San Jose River between Lac La Hache and Williams Lake where an estimated population of 50 wintered in 1939–40. This was reported to be the first instance of mallards remaining in the district through the winter.

General Conclusions from Banding Data

The recoveries of banded mallards show a wide summer dispersal of the species in British Columbia, Alberta, and Alaska and indicate the migration routes to these nesting grounds (Fig. 1). They show that a winter population is concentrated on a relatively small area along the lower Fraser River and Puget Sound, distributed with much less evidence of concentration through

all the western portion of Washington and Oregon, occupying portions of the eastern sections of these states and reaching its southern limit in north central California. The data indicate also that individual mallards migrate by the same routes to the same wintering grounds in successive years and that units of population maintain their association over a period of several years at least. They suggest that mallard populations in general are definite associations, nesting in the same locality, migrating together, and wintering together in the same areas from year to year.

TABLE IV

DISTRIBUTION OF TOTAL MALLARD RECOVERIES

Locality where recovered	Number	Locality where recovered	Number
Alaska	43	Montana	5
Alberta	68	North Dakota	1
British Columbia	2106	Nevada	1
California	11	Oregon	72
Idaho	9	Wyoming	1
Illinois	1	Washington	1055
Manitoba	1	Yukon Territory	10
Missouri	1	Northwest Territory	2
		TOTAL	3387

Reproduction

On the coast region mallards are paired in February or even earlier; copulation has been observed in January. They breed in their second year and there is no character apparent in life by which second year males can be distinguished from older males. Some associate in pairs so early as October; this is general in early spring prior to migration and flocks of transients composed chiefly of mated birds frequently have been observed. There is one record of male and female banded at Station 1 on February 12, 1934, and recovered the same spring at Fort Vermilion, Alta. Other records of male and female banded on the same day and recovered at or near the same place a year or more later, Table XIV, are taken to indicate a continued association of population units rather than of mated birds.

Courtship behaviour, as witnessed in March amongst a flock swimming on a small lake, consisted of vigorous bobbing by the male and a response in kind by the female. Occasionally a male made a quick forward movement and half rising from the water displayed the bright chestnut plumage of its breast. The courtship is of short duration and never reaches the excited pitch or the variety of movement that characterizes the courtship of some diving ducks. On the nesting grounds close companionship of a mated pair during the laying period is the rule but courtship has not been observed at this time.

Little information is available concerning the nesting of mallards in the coast region. In reference to southern Vancouver Island there is an exception-

ally early record of a brood of downy young, viz., Millstream, March 10, 1940 (I. McT. Cowan, personal letter). Another brood of downy young is recorded from "Extension Swamp" near Nanaimo, April 15, 1942 (A. L. Peake, personal letter).

Nesting is much later in northern parts of the coast region. At McClinton Creek, Queen Charlotte Islands, April 15–30, 1935, a population of 12 pairs were still associated in flocks during part of each day. So also was a population of approximately 12 pairs located along the Tlell River from a point near its mouth for a distance of five miles upstream. During the course of observation, May 4–14, 1935, these were seen in small flocks either in flight or resting on the stretches of shingle that sloped steeply to the river.

TABLE V

RECOVERIES OF MALLARDS, AUTUMN AND WINTER, ON COASTAL PLAIN, STATIONS 1, 2, 3

Locality where recovered	Current recoveries	Later years	Locality where recovered	Current recoveries	Later years
British Columbia—			Washington Counties—		
Mouth Fraser River	183	103	Whatcom	164	97
Sardis region	640	189	Skagit	190	229
Intermediate points	225	85	Snohomish	29	70
Pitt River region	171	91	San Juan	1	8
Harrison River region	41	17	Island	14	19
Vancouver Island	17	61	King	11	38
Gulf Islands	1	7			
			TOTALS	1687	1014

In the southern Okanagan Valley nesting commences about the first week in April, in the Nicola and Cariboo districts it is at least two weeks later. The following observations illustrate the pattern of behaviour at this time. In the Nicola district on April 24, 1939, all the mallards observed were paired. On certain lakes where the shoreline is open, so that it could be examined in detail, mated pairs rested on the beaches or on the water close to them. The distance separating two pairs was in no instance less than 200 yd., some were at much greater distances apart. A total of 37 counted on three lakes, April 24, 1941, consisted of 13 pairs and 11 single males. The latter were assumed to be on their territories while their mates were on their nests. The term "territory" is used here to identify the pond, or portion of stream, or area on lake shore usually occupied by a breeding pair. It is their feeding, breeding, and resting place; no behaviour that might be interpreted as territory defense has been observed. Very often such territory may be a long distance from the nest which the male apparently never visits.

The population as observed in the Cariboo district, April 27, 1939, consisted exclusively of paired birds; two weeks later, May 10–12, some territories appeared to be occupied by both male and female for long periods during the day while others under observation were occupied by males only. Heavy

floods occurred in June of that year and many nests were destroyed in consequence, a condition that might explain the several instances of females accompanied by males in eclipse recorded in early July. It was not determined if these pairs were nesting. During the period April 15–23, 1941, nearly every pond contained one or more pairs. At this time also flocks of transients numbering up to 20 or 30 and usually about equally divided as to sex were passing through. So late as May 26 a few still associated in pairs but the majority of males had already left the females. The following year nesting was later, thus at Springhouse, Cariboo, in early June small numbers could be identified as breeding and up to June 12, pairs were seen together on their territories. Possibly the latter may have represented a second laying following destruction of the first clutch.

Nesting

Mallards nest in many types of habitat from the river bottoms of the lower Okanagan Valley to the sedge-swamps and ponds in the subalpine forest. The following descriptions of nests illustrate the variety of sites chosen.

Monte Lake, Okanagan, May 1, 1940. Habitat, dry, open hillside above small pond; site under spreading branch of Rocky Mountain juniper; nest of down and vegetable debris; 9 eggs.

Okanagan Landing, May 5, 1915. Habitat, creek bottom, partly meadow, partly wooded with deciduous trees and shrubbery; site, small sedge marsh; nest of down concealed by dead alder branch; 10 eggs advanced in incubation.

Okanagan Landing, May 8, 1911. Habitat, same; site, grassy swale 50 yd. from stream; nest a 10-in. depression lined with down and broken pieces of round-stem bulrush, concealed by dead weed stalks; 11 eggs on point of hatching.

Okanagan Landing, May 10, 1917. Habitat, small mountain pond; site, three feet from water in small marsh of round-stem bulrush; nest of down mixed with small pieces of rotted bulrush; 10 eggs.

Westwick Lake, May 20, 1942. Habitat, round-stem bulrush marsh in lake; site, centre of large bulrush clump of previous year's growth beside open channel and about 100 ft. from shore; nest of short pieces of round-stem bulrush, some of which covered the eggs, and a small quantity of down; 9 eggs.

Springhouse, May 22, 1942. Habitat, 50-yd.-wide belt of round-stem bulrush marsh encircling small lake; site, thick clump of bulrush containing the yellowish stems of the previous year and the more weathered stems of an earlier year; nest of down and small quantity bulrush stems; 5 eggs.

Lac La Hache, May 28, 1942. Habitat, dry, wooded slope above lake; site, under drooping branch of Douglas fir 50 ft. from shore; nest of down mixed with fir needles and dry leaves; 10 eggs.

In the Peace River district in 1938 Cowan found the mallard to be the most abundant nesting duck and estimated the population at Swan Lake to be 100 pairs. Nests with full clutches of eggs were found from May 9 to May 31. Some were located under brush piles, in grass tufts near brush,

TABLE VI

RECOVERIES OF MALLARDS, AUTUMN AND WINTER, SOUTH, EAST, AND WEST OF COASTAL PLAIN, STATIONS 1, 2, 3

Locality where recovered	Current and first year	Later years	Locality where recovered	Current and first year	Later years
Western Washington Counties—			Eastern Washington Counties—		
Clallam	9	17	Okanogan		1
Jefferson		4	Chelan		2
Kitsap	1	2	Kittitas		3
Mason	1	3	Yakima	1	15
Grays Harbour	1	15	Klickitat		2
Pierce	4	13	Stevens		3
Thurston	1	8	Spokane	1	2
Lewis	3		Lincoln		2
Pacific	1	2	Whitman		5
Wahkiakum	1		Walla Walla		2
Cowlitz	1	5			
Clark	3	8	Eastern Oregon Counties—		
			Klamath	1	
Western Oregon Counties—			Umatilla		5
Clatsop	2	5	Lake		1
Columbia	4	11	Union		1
Washington	1				
Multnomah	3	11	Idaho State		8
Yamhill	2	2	California		8
Clackamas		1	Wyoming		1
Marion		4	Montana	1	3
Polk	1	1	North Dakota		1
Linn	1	2	Illinois		1
Benton		1	Manitoba		1
Lane	3		Missouri	1	
Coos		4			
			TOTALS	48	184

in open meadows, and at distances from water varying from six feet to almost two miles (1).

In the early part of the incubation period females leave the nest to bathe and feed once each day; usually the pond, or lake, or stream visited is the one on which mating had taken place earlier. At Elliot Lake, June 28, 1941, a female flew down hill over the tree tops and planed to the lake on down-curved wings. Evidently she came from a nest some considerable distance away. On the water she splashed with her wings, stood up and shook her wings then started to swim across the small lake. Every few minutes she dived, remained under several seconds and upon emerging splashed the water with her wings, shook them vigorously then dived again.

Survival of Young

Mallards hide their downy young very effectively and at an early age they are seen less often on the open water of ponds and lakes than are the young of other pond ducks. A great part of their early life is spent in thick grass cover, in brush thickets, and other places of concealment. If disturbed from such

situations the female may flush, fly a short distance, then drop to cover again while the young remain hidden.

The earliest records for young are: "Deadman's Lake" near Oliver, Okanagan, May 8, 1928, downy young; Rawlings Lake, May 28, 1922, brood approximately one week old; Lac La Hache, June 1, 1942, downy young; Swan Lake, Peace River, May 27, 1938, downy young (1). The latest record is Horse Lake, August 18, 1937, half-grown young.

The following are the earliest records of flying young: Swan Lake, Okanagan, July 11, 1932; 143 Mile, Cariboo, July 22, 1938; 102 Mile, Cariboo, July 23, 1938; Riske Creek, August 2, 1937. By the second week of August a large proportion of the season's increase has reached the flying stage.

The mortality in young appears to be less than in the young of other duck species. The average brood size in the Cariboo district is tabulated below. First figure = number of broods counted; second figure = average number of young in brood.

	June		July		August	
1936			5	5.8	7	5.8
1937			9	5.5	9	6.2
1938			38	6.5	2	6.5
1939			8	5		
1940	4	6				
1942	2	5.5				

Behaviour of Females and Young

Female mallards vigorously defend their young; the following observations show the variation of behaviour in this endeavour. Near Okanagan Landing on July 7, 1916, a female that was leading her brood across a grassy knoll between two ponds walked into the nearest one where she relaxed on the surface with outspread wings and neck outstretched on the water. In this position she struggled over the surface for a short distance then sank back as if exhausted. She repeated this action several times then flew to the adjacent pond where later she was discovered in the marginal brush with her half-grown young.

At Rawlings Lake, May 28, 1922, a female rushed down a steep, wooded bank that rises from the lake shore, followed by a number of small young that tumbled over each obstruction encountered. She swam out on the lake for 20 yd. or so, accompanied by a portion of the brood, then circled back to the shore half out of the water and beating the surface with her wings. She did this four times and on each occasion found one of the lagging young. After all the young had reached the water she led them toward the centre of the lake.

At Bridge Creek on July 17, 1937, a female was seen leading seven half-grown young into the marginal sedges. After they had disappeared in this

cover she swam out in front of a passing canoe and made a series of dives accompanied by much splashing, one dive following another in quick succession as she proceeded up stream.

On July 1, 1938, at Exeter Lake, Cariboo, two female mallards, accompanied by two quarter-grown young, were swimming along at the edge of the shoreward rushes. A few minutes later the young swam into the marsh, where no doubt the remaining members of the broods were concealed, and the two adult females joined a female ring-necked duck, *Nyroca collaris*, that was flapping over the water in front of her brood of seven small young. All three females proceeded to act in the same manner. They pushed themselves along the surface thrashing the water with their wings, sometimes in advance of, sometimes making a complete circle about the running brood. Somewhat later another female mallard suddenly rose from the edge of a *Carex* marsh, where probably her brood was hiding, then quickly dropped to the water again in front of the observer's canoe. She swam ahead for 20 yd. or so, wings churning the water and head submerged so that only her back was visible, then rose and making a wide circle flew back to the marsh.

At 105 Mile Lake, June 16, 1942, a female mallard lay partly submerged and motionless on the water near the outer edge of a round-stem bulrush marsh. When approached by a canoe to a distance of 15 ft. she flapped out from the marsh moving in this manner for about 30 yd. then taking flight. She alighted 300 yd. or so from the shore, rested there for a short time then made a series of short flights sometimes flapping along the surface for a short distance after alighting. The brood, undoubtedly concealed in the rushes, was not seen.

Behaviour of Postbreeding Males

Postbreeding males, still in full breeding plumage, commence to flock soon after incubation has begun and territorial segregation then ceases. One instance of a male accompanying a female with downy young—"Deadman's Lake", May 28, 1928— is the only exception noted and this may have been, and probably was, a fortuitous association. An instance of three males remaining together all day at Springhouse, Cariboo, May 21, 1942, represents the earliest date upon which postbreeding association of males came under observation. Three females, believed to have been mated with these particular males, were incubating eggs in the vicinity at this time and so far as could be learned had ceased visiting their respective mates. By May 28 the original band of three males had increased to 11. Other examples of postbreeding males in full breeding plumage are: 74 Mile, Cariboo, May 26, 1941—30; 103 Mile Lake, Cariboo, May 26, 1941—20; Westwick Lake, May 30, 1941—35; Swan Lake, Okanagan, June 4, 1938—38; 70 Mile, Cariboo, June 7, 1937—19.

After several weeks, spent usually on the more open waters, the males commence to frequent areas provided with dense cover, such as brush-fringed ponds and small lakes, willow swamps, and marshes of round-stem bulrush.

Although some such places may contain large numbers of males they no longer associate in flocks; they have become shy and it is difficult to flush them. Round-stem bulrush marshes are favoured resorts at this time and show evidence of occupation even if the birds are not seen. Thus at Westwick Lake in early June, 1941, practically all the muskrat houses, old grebes' nests, piles of rotted vegetation, and other small resting places in the marsh were strewn with mallard feathers, chiefly those from flank and chest but including also wing primaries and secondaries. A number of moulting mallards were found here at this time. When alarmed those still capable of flight rose heavily and after flying a short distance dropped into the rushes again while those that were flightless could be heard and occasionally seen in the thick cover. Sometimes moulting males are discovered in situations where the cover affords less effective concealment. Thus at Horse Lake, July 24, 1936, one swam through an open patch of rushes in deep water in advance of a moving canoe. It made several unsuccessful attempts to rise, then finally did so and flew low over the water for half a mile or so. The earliest date for a male in full eclipse, or in nearly full eclipse, is June 9, 1930, when one, with wing feathers partially moulted, was flushed from the marsh at Swan Lake, Okanagan.

TABLE VII

SELECTED CURRENT RECOVERIES OF MALLARDS SOUTH, EAST, AND WEST OF COASTAL PLAIN, STATION 1

Date banded	Locality where recovered	Date recovered
Nov. 9, 1928	Columbia Co., Oregon	Nov. 18, 1928
Nov. 4, 1930	Linn Co., Oregon	Nov. 23, 1930
Nov. 9, 1931	Pierce Co., Wash.	Nov. 16, 1931
Nov. 11, 1931	Polk Co., Oregon	Dec. 13, 1931
Oct. 19, 1932	Pierce Co., Wash.	Oct. 23, 1932
Nov. 6, 1932	Multnomah Co., Oregon	Nov. 9, 1932
Nov. 8, 1933	Clallam Co., Wash.	Nov. 26, 1933
Dec. 10, 1933	Clallam Co., Wash.	Dec. 15, 1933
Oct. 21, 1933	Grays Hbr. Co., Wash.	Nov. 8, 1933
Nov. 8, 1933	Multnomah Co., Oregon	Nov. 25, 1933
Oct. 29, 1934	Cowlitz Co., Wash.	Nov. 11, 1934
Nov. 3, 1934	Clatsop Co., Oregon	Nov. 23, 1934
Nov. 3, 1934	Columbia Co., Oregon	Nov. 18, 1934
Nov. 22, 1934	Clallam Co., Wash.	Dec. 22, 1934
Oct. 23, 1936	Wahkiakum Co., Wash.	Nov. 5, 1936
Oct. 25, 1936	Lane Co., Oregon	Nov. 8, 1936
Nov. 2, 1937	Klamath Co., Wash.	Nov. 7, 1937
Nov. 2, 1937	Kitsap Co., Wash.	Dec. 12, 1937

In early July males that have renewed their flight feathers commence to appear on the more open waters. July 1, 1938, is the earliest date for such in the Cariboo district. In that year two other early appearances were recorded at Williams Lake on July 4 and July 5. On July 11, 1940, seven single birds were flushed from the marshes on Tatton Lake. Later in the month the males again commence to flock. Examples showing the size of these gather-

ings are: McKinley Lake, July 20, 1938—200±; Mirage Lake, August 1, 1938—70; 150 Mile Lake, August 2, 1937—20.

As the summer passes flocks of flying eclipse males are joined by females and young. The following counts are typical of these mixed associations as they occur in the Cariboo district: Buffalo Lake, August 7, 1936—190±; 103 Mile Lake, August 8, 1936—100±; Cummings Lake, August 10, 1938—300±; 103 Mile Lake, August 20, 1937—200±.

Summary

It can be said in brief summary that in the more southern districts of interior British Columbia mallards are mated in March, they associate generally in pairs during the laying period in April and May but sometimes assemble in small flocks. After incubation begins the males, still in full breeding plumage, gather in flocks; subsequently they disperse and go into eclipse. When the flight feathers are renewed a large percentage again assemble in flocks while the remainder remain solitary. Later some of these flocks are joined by females and flying young.

Restrictive Factors

The mallard is the duck most highly regarded as an object of sport and for food, consequently the hunting pressure is greater on this species than on other ducks. In some localities hunters attempt with frequent success to restrict their daily bag to mallards and if possible to males only. The males, because of their larger size are more sought after than the females; nevertheless on the basis of banding records the number of females shot is greater than the number of males. In a total of 2576 banded birds shot, 1299 were female and 1277 male.

Lead poisoning, caused by the ingestion of lead shot, is a restrictive factor of unknown proportion. It seldom is apparent in the interior of the province but is of common occurrence in the lower Fraser Valley where hunting is more concentrated and hunting grounds contain great quantities of shot that have accumulated through many years. Lead poisoning is most evident in dry years when low water has exposed areas of mud flat and lake bottom that at other times, because of the depth of water that covers them, are not accessible to mallards. The stomachs of 12 mallards in a total of 90 shot in this region during November, 1934, contained shot. Whether or not these had the symptoms of lead poisoning was not determined as the bodies were not available for examination. There is some evidence that mallards may develop resistance to lead poisoning and this appeared to be so in three from Pitt Meadows each of which contained shot in the stomach. In two the pellets were worn down to disks which indicated that the birds had been absorbing an amount of lead that in most ducks would constitute a lethal dose. All were in normal condition and showed none of the symptoms associated with lead poisoning.

The flooding of mallard nests in spring and, conversely, summer droughts that restrict food on the nesting grounds are factors that operate unfavourably. So also is the tendency of mallards to winter in districts where subzero temperature greatly restricts the food supply.

TABLE VIII

RECOVERIES OF TRANSIENT MALLARDS NORTH OF COASTAL PLAIN, STATIONS 1, 2, 3

Locality where recovered	Number of recoveries			
	First spring	Later years	First autumn	Later years
British Columbia—				
Mainland coast			10	3
Nicola region	1		8	7
Kamloops region			8	5
Okanagan region			4	6
Cariboo region	5	3	19	16
Chilcotin region	3	5	7	7
Central region	8	9	12	15
Peace River region	2		5	2
Northern region	2	1		1
Grand Forks				1
West Kootenay				1
Revelstoke				1
East Kootenay			1	
Donald				1
Alberta—				
Southern region			4	4
Central region	2	1	7	1
Peace River region		3	18	12
Northern region	5	4	6	1
Yukon Territory	5	1		4
Northwest Territories	1		1	
Alaska—				
Eastern region	2	1	8	1
Western region	3	2	7	1
Interior region	4	3	2	8
TOTALS	43	33	127	98

Few instances of predation have been observed. In the Okanagan Valley, December 19, 1935, a gyrfalcon, *Falco rusticolus*, was shot while it was in pursuit of a female mallard. Examination of the falcon's crop showed it recently had eaten parts of a mallard. Near the same place on December 19, 1935, a goshawk, *Astur atricapillus*, was watched in an unsuccessful attempt to capture a mallard out of a large flock that circled about a stubble field. At White Horse Lake, July 27, 1938, the remains of a male mallard in eclipse plumage, lying on a flattened tussock of *Carex* on the muskeg shore, was believed to represent predation by a horned owl, *Bubo virginianus*.

The destruction of mallards' eggs by crows is a probable restrictive factor about which little is known. Concerning mallards of the Peace River district

Cowan reports that in 1938 they suffered heavily from this cause (1). Only once has destruction of mallard eggs by crows been observed by the writer and this was of an unusual nature. At Monte Lake, Okanagan, on May 1, 1940, a female mallard was flushed from a nest, well concealed under the branches of a Rocky Mountain juniper and containing 12 eggs. On May 9, three eggs lay on the ground a foot or so from the nest and from each the contents had been removed through a puncture in the side—undoubtedly the work of a crow. The remaining nine eggs were cold, the nest disarranged and swarms of red ants fed on the liquid that covered both the punctured eggs and those in the nest. The female was not seen and it was assumed she had deserted. On May 13, she was again on the nest which now contained only seven eggs. The nest had been remade, the eggs were half-hidden in down, and all traces of broken eggs and egg debris had disappeared.

In spite of a high degree of hunting pressure, and whatever other restrictive factors may operate, the recuperative power of the species is such that populations maintain their numbers and under extra favourable conditions increase. Because of its adaptibility in the matter of food and nesting requirements it has on the whole thrived under agricultural expansion in British Columbia. While in some areas drainage and subsequent cultivation have destroyed the source of one food supply, another of a different type in the form of grain, grasses, and weed seeds has taken its place. To this the species quickly adapted itself as it has done also to a changed type of cover. The clearing and subsequent cultivation of brush lands has produced similar results elsewhere. Most conspicuous, however, is the modification brought about in the interior of the province by the replacement of grasslands by grain fields thus providing an additional and highly nutritious type of food and a higher population potential.

Sex Ratio

A total of 13,959 mallards for which sex records are available was banded at Station 1 during the period 1928–40 (Table III). Of these 6381, or 45.7%, were male, and 7578, or 54.3%, female. The number of females exceeded the number of males in each banding period except those of November–December, 1931, and November, 1939–March, 1940, when the reverse was the case. Banding recoveries show that more females are shot than males; in a 12-yr. period the numbers recorded were 1299 females and 1277 males. Thus hunting probably is not a factor in the sexual unbalance suggested by these figures.

Food and Feeding Habits

Coast Region

During autumn and winter the feeding grounds of one mallard population in the lower Fraser Valley is centred about the hay meadows and cultivated fields and the drainage ditches, sloughs, and swamps of this habitat. Food in the stomachs of specimens collected in this region was composed exclusively

of seeds of native plants and introduced weeds, grain and miscellaneous vegetation including a large percentage of grass leaves and fibres. In the Pitt Meadows the seeds of smartweed, *Polygonum hydropiper*, and other members of the Polygonaceae are eaten in large quantities and this diet is said to impart a distinctive flavour to their flesh. Another population feeds in the salt marshes and on tidal flats; stomachs from specimens taken in this habitat contained a variety of small marine animals, together with marine algae and seeds of various plants. A third population follows the spawning runs of salmon, which continue from October to January, and feeds exclusively on salmon eggs and salmon flesh.

TABLE IX

SELECTED RECOVERIES OF MALLARDS, FIRST SPRING, STATIONS 1, 2, 3

Date banded	Locality where recovered	Date recovered
Dec. 7, 1938	Lower Nicola, B.C.	June 5, 1939
Feb. 12, 1934	Lac La Hache, B.C.	April 30, 1934
Feb. 25, 1934	Horsefly, B.C.	April 6, 1934
Feb. 1, 1934	Horsefly, B.C.	May 4, 1934
Feb. 25, 1934	Woodpecker, B.C.	April 21, 1934
Nov. 3, 1932	Baker Creek, Quesnel, B.C.	April 27, 1932
Jan. 28, 1934	Kleena Kleen, B.C.	Spring, 1934
Nov. 11, 1933	Alexis Creek, B.C.	Spring, 1934
Feb. 20, 1934	Stuart Lake, B.C.	April 26, 1934
Nov. 11, 1932	Stuart Lake, B.C.	May 22, 1933
Nov. 19, 1933	Crooked River, B.C.	April 14, 1934
Feb. 20, 1934	Telkwa, B.C.	May 6, 1934
Feb. 19, 1934	Fort Fraser, B.C.	April 15, 1934
Mar. 4, 1939	Ootsa Lake, B.C.	April 15, 1939
Jan. 9, 1936	Dawson Creek, B.C.	April 30, 1936
Dec. 23, 1939	Moberly Lake, B.C.	April 14, 1940
Dec. 27, 1935	McDames Creek, B.C.	June, 1936
Mar. 14, 1933	Tp. 62, R. 12, W.4, Alta.	Spring, 1933
April 13, 1939	Mount Valley, Alta.	April, 1939
Feb. 12, 1934	Fort Vermilion, Alta.	May 14, 1934
Feb. 12, 1934	Fort Vermilion, Alta.	Spring, 1934
Jan. 6, 1940	Belloy, Alta.	April 10, 1940
Nov. 18, 1933	Athabasca, Alta.	May 9, 1934
Mar. 10, 1934	White Horse, Yukon	May 2, 1934
Oct. 21, 1933	White Horse, Yukon	May 15, 1934
Nov. 13, 1934	Carmacks, Yukon	May 10, 1935
Oct. 26, 1932	Kloo Lake, Yukon	May 10, 1933
Dec. 15, 1938	Liard River, N.W.T.	May 10, 1939
Mar. 10, 1934	Kwiguk, Alaska	May 21, 1934
April 17, 1934	Galena, Alaska	May 21, 1934
Feb. 17, 1934	Fort Yukon, Alaska	May 25, 1934
Feb. 17, 1934	Fort Yukon, Alaska	May 25, 1934
Nov. 19, 1933	Eagle, Alaska	May 2, 1934
Nov. 19, 1933	Nushagak, Alaska	April 1, 1934
Dec. 13, 1935	Yukutat, Alaska	May 16, 1936

In some localities such as Boundary Bay and the mouth of the Fraser River the birds congregate on the sea in the daytime and visit the fields to feed in morning and evening. After the shooting season has been in progress for a short time night-feeding becomes general and the evening flight does not

commence until the approach of darkness. This applies also at Pitt Lake and at other points along the Fraser River some distance inland where mallards concentrate in daytime. There is no evidence, however, that the population that uses the sea as a resting place feeds on the food available there. During spells of cold weather when surface water on the fields is frozen the population inhabiting the littoral is increased by an influx of individuals representing a part of the population that feeds on the fields, but so long as mild weather continues and small water areas remain open these populations continue to feed in these several separate habitats. In the analysis of stomach contents almost without exception the items present can be used to designate the general area where the specimen was taken. Only in one bird was a possible exception noted. In this, the stomach contained a large amount of vegetation and a fragment of organic material identified as salmon flesh. These findings would seem to afford further evidence that mallards associate in definite populations that are maintained from year to year.

On southern Vancouver Island a winter population makes daily flights from the sea to the flooded fields in the bottom lands. Here they find a variety of food including the seeds of many species of weeds and they have been seen feeding on potatoes that flooding had partially uncovered. In winters cold enough to freeze the water on the fields the birds remain close to the sea where they frequent bulrush and sedge marshes and the open tide flats. Here they find seeds of various kinds, mostly obtained on the ground or on the surface of the water, and on the tide flats they feed upon algae, molluscs, crustaceans, and other small animals exposed by a receding tide.

At Clements Creek, a tributary to Henderson Lake on the west coast of Vancouver Island, 4 to 15 mallards were observed daily during November and early December, 1922, feeding on salmon eggs. They fed by "dipping" in the shallow parts of the creek and after freshets had receded found many stranded eggs along the stream banks and in shallow pools in the brush back from the main stream channels (5). This habit is general on all the salmon streams of the coast. Mallards feed also on the flesh of spawned-out salmon and the diet of salmon eggs and salmon flesh, that usually is partly decomposed, imparts a rank flavour to the birds that consume it.

Interior Region

In the northern Okanagan Valley during autumn one mallard population feeds almost exclusively in the grain fields. Here, as in the prairie provinces, feeding in the grain fields occurred seldom, if ever, during the early days of settlement. The habit developed slowly with the expansion of acreage sown to grain and later suddenly reached large proportions. In the Vernon district few mallards visited the stubble prior to 1930 but by the autumn of 1932 this manner of feeding had become a fixed habit. Flocks fed in the fields chiefly in the morning and evening, resting during part of the day on the open water of Okanagan Lake and to a lesser extent on Swan Lake. The following is characteristic of their behaviour.

From a hilltop north of Swan Lake on the evening of November 18, 1932, flocks of mallards, mostly invisible in a thick fog, were heard flying high in the air and alighting in a stubble field close to the lake. Occasionally a flock loomed up suddenly at close range but for the most part the birds were invisible. Overhead was heard the soft call of the males and less often, from flocks at high altitudes and flying very fast, a quick, sharp report like a clap of the hands, the source of which could not be determined. From the distant fields shrouded in fog came the *kak, kak, kak* sound of feeding birds. On the following morning flocks commenced to arrive in the fields before daylight; the whistling of wings and the tearing sound produced by masses of swiftly moving birds could be heard from all directions. Later in the early light a flock of 400 or more began circling the field high up, at times in a dense

TABLE X

RECOVERY OF MALLARDS BANDED AT STATION 4, VASEAUX LAKE

Locality where recovered	Current and first year	Later years	Locality where recovered	Current and first year	Later years
Osoyoos to Penticton, B.C.	18	27	Grant Co., Wash.		1
Naramata, B.C.	1		Spokane Co., Wash.	1	
Kelowna, B.C.	1	1	Walla Walla Co., Wash.	1	
Mara, B.C.	1		Whitman Co., Wash.	1	
Knouff Lake, B.C.		1	Lincoln Co., Wash.		1
Similkameen Valley, B.C.	1	1	Nez Perce Co., Idaho	1	
Coastal Plain, B.C.		1	Columbia Co., Oregon	1	
Coastal Plain, Wash.		1	Multnomah Co., Oregon		1
Okanogan Co., Wash.	1		Polk Co., Oregon	1	
Chelan Co., Wash.	1		Yukon Territory		1
Yakima Co., Wash.	1	1	TOTALS	31	37

flock, then spreading out, then closing their ranks again as they wheeled, rose, or descended in fast flight. Flocks arrived and departed during the entire day but less often between 1:30 and 3:00 P.M. Large flocks, flying at great speed, would circle the fields numerous times in ever lessening circles, descending lower and lower with each circle, sometimes to disappear in the mist that hung low over the surrounding hills. Finally all alighted, some beside a flock of Canada geese that were feeding near the centre of the field a mile away, others at more distant points. It was estimated that 3500 visited the field during the day. Later in the same month a flock numbering $2000\pm$ was seen rising from this field. The birds first flew low over the stubble in a massed flock that gradually lengthened as they rose higher and higher on a westward course that finally took them over the ridge toward Okanagan Lake. In the late autumn of 1941 and of 1942 the number of mallards in the Vernon district was at least equal to and probably exceeded the numbers present 10 yr. earlier. A larger number frequented Swan Lake in daytime where they rested in large flocks on the open water, often associated with a raft of diving ducks, baldpates, and coots.

As stated earlier the autumn concentration of mallards in the northern Okanagan district, which had resulted from the development of the grain-eating habit, greatly increased the number of the wintering population. Recent mild winters, with light precipitation, provided a condition in which waste grain on the fields was always available in sufficient amounts to support them. Thus at Kelowna in the winter of 1939–40 the grain fields continued bare and some 2000 mallards remained there as did a large number in the Vernon district. The latter also obtained most of their food from the stubble fields but one flock numbering over 300 made daily visits to a farm where hogs were being raised and here they fed on oats that were thrown into the hog pens. These mallards became tamed to such an extent that they would not take flight when closely approached. In several other years, however, a sudden drop to subzero temperature, accompanied by snow, made grain in the fields inaccessible so that mallards were hard pressed for food and many did not survive.

Distinct from this concentrated population of grain-feeding mallards are other populations of smaller size that remain in the small streams, ponds, sloughs, and marshes throughout the district. At Swan Lake a number frequent the shoreline marshes and apparently do not mix with the grain-feeding population that spends part of the day on the open waters of this lake. When disturbed the latter leave the lake while those inhabiting the shoreline usually fly to another part of the marsh. The marsh-dwelling mallards feed chiefly on aquatic insect larvae and seeds of aquatic plants; specimens taken from this habitat rarely contain grain (4).

To what extent salmon eggs are consumed on the spawning streams of the interior is not known. The one mallard secured from Babine Lake contained sockeye salmon eggs and there is evidence that on Okanagan Lake the eggs of kokanee, *Salmo nerka kennerleyi*, are eaten. Here on numerous occasions in November flocks of mallards have been seen on the stony beaches along which kokanee had spawned a few weeks earlier. As no food other than dead kokanees and washed-out eggs was available it can be assumed that either or both were being eaten.

Food Summaries

In the following section a summary is given of the food eaten by 118 mallards collected in the coast region and 100 collected in the interior of the province. The figure following the month indicates the number of specimens examined.

Coast Region

Fresh Water Habitat

Colquitz, December, 1; Langford Lake, November, 1; Thetis Lake, December, 1; Cowichan River, November, 1; Mervale, December, 1; Quinsome Lake, January, 5; Henderson Lake, November, 6.

Salmon. One stomach from Henderson Lake contained a few salmon bones as the sole item. In another from Quinsome Lake salmon flesh composed 50% of the contents.

Salmon eggs. Seven specimens contained salmon eggs as the exclusive item; in two others this represented 20 and 80% respectively of the stomach contents. In quantity the number varied from two in one bird to 451 in another. Those in the specimens from Henderson Lake were chiefly sockeye salmon; those in specimens from Quinsome Lake were identified as probably chum salmon, *Oncorhynchus keta*.

Fishes. A stomach from Henderson Lake contained bones of a small fish not identified as to species.

Aquatic insects. Caddis larvae composed 99% of the food eaten by a specimen from Thetis Lake and was a minor percentage in another from Henderson Lake. A small quantity of insect debris was contained in a stomach from Cowichan River.

TABLE XI

RECOVERY OF MALLARDS BANDED AT STATION 5, BUFFALO LAKE

Locality where recovered	Current and first year	Later years	Locality where recovered	Current and first year	Later years
Buffalo Lake region	10	5	Coastal Plain, British Columbia	3	1
Southern interior, British Columbia	4	1	Coastal Plain, Vancouver Island	4	
Eastern Washington Counties—			Coastal Plain, Washington	6	5
Okanogan	1		Western Washington Counties—		
Yakima	2	2	Pierce	1	
Lincoln	3		Mason	1	
Walla Walla	2		Lewis		2
Benton	1		Clark	2	
Eastern Oregon Counties—			Grays Harbour	2	1
Klamath	1		Western Oregon Counties—		
Umatilla	1	1	Columbia	2	1
Montana State	1		Multnomah	3	
Nevada State		1	Tillamook		1
California State	3		Benton	2	
			TOTALS	55	21

Seeds. Except in one stomach from Langford Lake, where seeds were the exclusive item, this food represented only small percentages and occurred in a total of four stomachs. Species identified were *Potamogeton pectinatus*, *Sparganium* sp., *Menyanthes trifoliata*, and *Galium* sp.

Grain. Oak husks were present as a small percentage in two stomachs.

Miscellaneous vegetation. Plant debris, including in one specimen pieces of *Equisetum*, constituted 80%, 90%, and 100% of the contents of three and occurred as a minor item in one other stomach.

Lower Fraser Valley, November, 90.

Seeds. Seeds, chiefly of weeds and aquatic plants, were present in all but three of the 90 stomachs and in 48 composed 90 to 100% of the contents. The species of greatest importance in times of occurrence and volumetric

percentage was wild oat, *Avena fatua*, representing 90 to 100% of the food in 27 stomachs and occurring 41 times. Other seeds were present the number of times indicated, viz.; *Typha latifolia*, 1; *Potamogeton heterophyllus*, 1; *P. pusillus*, 1; *P. foliosus*, 25; *P. pectinatus*, 6; *Ceratophyllum demersum*, 2; *Echinochloa crusgalli*, 2; *Carex exsiccata*, 4; *Carex* sp., 5; *Scirpus americanus*, 1; *S. acutus*, 19; *Eleocharis palustris*, 12; *Polygonum convolvulus*, 19; *P. aviculare*, 1; *P. amphibium* or *Muhlenbergii*, 9; *P. lapathafolium*, 30; *P. hydropiper*, 2; *P. acre*, 2; *P. persicaria*, 5; *P. hydropiperoides*, 10; *Chenopodium* sp., 4; *Atriplex* sp., 1; *Thlaspi arvense*, 4; *Nestia peniculata*, 3; *Rosa* sp., 1; *Cornus occidentalis*, 1; *Mentha* sp., 3; *Galium* sp., 1.

Grain. Twelve stomachs contained wheat or oats representing 50% or more of the contents of three and small percentages in the remainder.

Miscellaneous vegetation. Grass leaves and unidentified vegetable matter was present in 41 and composed 90 to 100% of the total food in 21 stomachs.

Pitt Meadows, December, 6.

Seeds. Seeds, chiefly of aquatic plants, were the exclusive item in all specimens, *Carex vesicaria* being the species of first importance. Other seeds identified were *Eleocharis obtusa*, *Polygonum hydropiper*, and *Menyanthes trifoliata*.

Salt Water Habitat

Boundary Bay, November, 1, December, 1; Departure Bay, January, 2; Seal Island, January, 1.

Crustaceans. A small shore crab, *Hemigrapsus* sp., was the chief item in one, and a mixture of shore crab and amphipod debris composed 80% of the food in another stomach.

Mollusca. Small gastropods, including whole *Littorina scutulata* but represented chiefly by shell fragments of unidentified species, were present in three stomachs.

Algae. Pieces of unidentified algae constituted 95% and 50% of the food in two stomachs.

Seeds. Seeds of *Atriplex hastata* composed 40% of the food eaten by one specimen from Boundary Bay and seeds of *Eleocharis* sp. were present in another from Departure Bay.

Interior Region

Cariboo

Lone Butte Lake, September, 1; 105 Mile Lake, September, 2; Tatton Lake, September, 1; Slough, 108 Mile, September, 1, October, 1; 122 Mile Creek, September, 3.

Amphipods. Amphipods composed 99% of the food in one specimen from 122 Mile Creek.

Aquatic insects. Dragonfly nymphs represented 10% of the total food in one stomach which contained also remains of crickets, members of the Orthoptera, and adult chironomids totalling 15%. In two stomachs were traces of corixids and in two others insect debris was a minor item.

Molluscs. Fragments of small gastropods were present in five stomachs, in one comprising 50%, in another 60%, of the total contents and in three others representing a minor item.

Miscellaneous animals. Bryozoan statoblasts were a minor item in one stomach.

Seeds. Seeds of aquatic plants were present in all but one of the nine stomachs from this region and comprised the sole item in one, the chief item in two, and an important constituent of the food in three others. The species most frequently represented were *Potamogeton pectinatus*, *P. pusillus*, and *Scirpus acutus*. Other genera represented were *Eleocharis*, *Myriophyllum*, *Sparganium*, and *Polygonum*.

Miscellaneous vegetation. Grass stems were the sole item in one and occurred in a second stomach. Rootlets and leaves of a *Potamogeton* was the only other identifiable item in three stomachs in which miscellaneous vegetation composed 15, 89, and 98% respectively of the total contents.

Okanagan

Cherry Creek, July, 1; Trinity Valley, October, 2; Vernon Commonage ponds, September, 2.

Aquatic insects. Dragonfly nymphs composed 6% in one and 3% in another stomach, both of which also contained three or more corixids and in one an adult beetle, *Dystiscus* sp.

Miscellaneous animals. One specimen contained five, another approximately 900, bryozoan statoblasts; in the latter this item represented 48% of the stomach contents.

Algae. Three stomachs contained *Chara* oospores, in one representing 2%, in another 5%, and in a third combined with *Chara* branches, 99% of the total contents.

Seeds. In one stomach 66 seeds of *Potamogeton pectinatus* composed 30%, and in another 120 seeds composed 35%, of the contents. Other seeds present in comparable bulk were *Potamogeton foliosus*, *P. pusillus*, *Sparganium* sp., *Nuphar polysephalum*, *Polygonum lapathafolium*, and *Ceratophyllum demersum*.

Swan Lake, September, 15, October, 45, November, 23, December, 2.

Aquatic insects. Aquatic insects were present in 65 stomachs, the most important in volumetric percentage being *Odonata* which was represented by 42 occurrences of dragonfly nymphs and 11 of damsel fly nymphs with a total percentage volume of 22.94%. In 16 birds the former composed 50% or more of the total stomach contents. Corixids, including *Arctocorixa laevigata* Uhl, were of next importance, occurring in 50 stomachs with a

total percentage volume of 18.45% and in 11 representing 50% or more of the food eaten. Other insects were represented the number of times indicated, viz.: micro-tricoptera, 2; fly larva, 1; chironomid larvae, 3; Cyrinidae, 4.

Coleoptera. Insect debris included in five birds the elytra of terrestrial beetles representing a minor percentage in each case.

Molluscs. Gastropods, represented by broken shells and debris and constituting small percentages usually less than 1%, occurred 13 times. A small bivalve, *Pisidium* sp., also was detected in one stomach.

Miscellaneous animals. There were two occurrences in this category, in one case several amphipods, in another a leech had been eaten.

TABLE XII

AUTUMN DISPERSAL OF MALLARDS FROM STATION 5, BUFFALO LAKE

Date banded	Locality where recovered.	Date recovered
Migration to Coastal Plain and Puget Sound		
Sept. 22, 1932	Chase, B.C.	Dec. 14, 1932
Sept. 20, 1932	Sardis Region, B.C.	Nov. 6, 1932
Oct. 9, 1933	Sardis Region, B.C.	Oct. 31, 1933
Sept. 23, 1933	Pitt River, B.C.	Dec. 31, 1933
Sept. 19, 1933	Barclay Sound, V.I., B.C.	Nov. 18, 1933
Sept. 25, 1933	Duncan, V.I., B.C.	Dec. 12, 1933
Oct. 14, 1933	Saanich, V.I., B.C.	Oct. 31, 1933
Oct. 15, 1933	Quamichan Lake, V.I., B.C.	Dec. 28, 1933
Sept. 24, 1932	Whatcom Co., Wash.	Dec. 3, 1932
Aug. 30, 1932	Skagit Co., Wash.	Nov. 20, 1932
Sept. 24, 1932	Skagit Co., Wash.	Nov. 27, 1932
Oct. 15, 1933	Skagit Co., Wash.	Dec. 6, 1933
Sept. 25, 1933	King Co., Wash.	Dec. 5, 1933
Sept. 14, 1932	Island Co., Wash.	Dec. 15, 1932
Sept. 6, 1932	Grays Hbr. Co., Wash.	Dec. 14, 1932
Sept. 24, 1932	Grays Hbr. Co., Wash.	Dec. 15, 1932
Oct. 1, 1932	Pierce Co., Wash.	Nov. 17, 1932
Sept. 14, 1932	Mason Co., Wash.	Oct. 29, 1932
Inland migration by Columbia River		
Sept. 14, 1933	Kamloops, B.C.	Oct. 31, 1933
Oct. 6, 1932	Armstrong, B.C.	Dec. 21, 1932
Sept. 13, 1932	Vernon, B.C.	Nov. 27, 1932
Sept. 10, 1932	Okanogan Co., Wash.	Dec. 4, 1932
Sept. 12, 1933	Lincoln Co., Wash.	Oct. 25, 1933
Sept. 30, 1933	Lincoln Co., Wash.	Nov. 6, 1933
Sept. 22, 1932	Yakima Co., Wash.	Dec. 4, 1932
Oct. 15, 1932	Yakima Co., Wash.	Dec. 15, 1932
Sept. 15, 1933	Walla Walla Co., Wash.	Dec. 3, 1933
Sept. 23, 1933	Walla Walla Co., Wash.	Dec. 6, 1933
Sept. 22, 1932	Clark Co., Wash.	Nov. 13, 1932
Sept. 30, 1933	Clark Co., Wash.	Nov. 2, 1933
Sept. 14, 1933	Columbia Co., Wash.	Dec. 6, 1933
Aug. 30, 1932	Benton Co., Wash.	Nov. 6, 1932
Sept. 23, 1933	Multnomah Co., Wash.	Nov. 5, 1933
Sept. 27, 1933	Multnomah Co., Wash.	Nov. 12, 1933
Sept. 19, 1933	Los Benos, Calif.	Nov. 29, 1933
Sept. 22, 1932	Oakley, Calif.	Nov. 22, 1932
Sept. 28, 1932	Inyo Co., Calif.	Dec. 26, 1932

TABLE XIII

TRAP RECOVERIES OF BANDED MALLARDS

Station banded	Date banded	Station where recovered	Dates recovered
1	Nov. 12, 1930	3	Oct. 17, 1934
4	Nov. 9, 1932	4	Nov. 8, 1933
4	Nov. 10, 1932	4	Nov. 7, 1933
4	Nov. 10, 1932	4	Nov. 7, 1933
3	Nov. 27, 1932	3	Dec. 3, 1933
1	Nov. 30, 1932	3	Dec. 3, 1933
3	Dec. 18, 1932	3	Dec. 3, 1933
3	Oct. 14, 1933	1	Nov. 26, 1934
1	Nov. 3, 1933	2	April 15, 1934
1	Nov. 19, 1933	1	Nov. 26, 1933
1	Dec. 7, 1933	1	Jan. 16, 22, 1936
1	Feb. 3, 1934	1	Jan. 25, 1936
1	Mar. 8, 1934	1	Jan. 22, 1936
2	April 18, 1934	1	Nov. 27, 1934
1	Jan. 14, 1936	1	April 7, 1937
1	Dec. 28, 1936	1	Mar. 24, 1939
1	Nov. 21, 1938	1	Mar. 8, Dec. 25, 1939
1	Nov. 24, 1938	1	Dec. 9, 1939
1	Dec. 7, 1938	1	Dec. 5, 1939
1	Dec. 12, 1938	1	Nov. 17, Dec. 7, 1939
1	Dec. 19, 1938	1	Dec. 22, 1939
1	Dec. 19, 1938	1	Dec. 14, 1939
1	Dec. 22, 1938	1	Dec. 30, 1939
1	Dec. 22, 1938	1	Nov. 15, 1939
1	Jan. 24, 1939	1	Dec. 17, 1939, Jan. 7, 1940
1	Jan. 27, 1939	1	Mar. 15, 1940
1	Feb. 1, 1939	1	Jan. 5, 10, 1940
1	Feb. 22, 1939	1	Mar. 23, 1940
1	Feb. 23, 1939	1	April 8, 1939, Nov. 14, 1940
1	Mar. 8, 1939	1	Dec. 9, 1939, Mar. 15, 1940
1	Dec. 24, 1939	1	Dec. 29, 1941
Big Suamico, Wis.	Oct. 31, 1929	3	Oct. 21, 1934
Oak Harbour, Wash.	Dec. 8, 1937	1	Feb. 20, 23, 1939
1	Nov. 18, 1932	Stn. Athabasca, Alta.	May 9, 1934

Algae. One bird had eaten a small amount of filamentous algae, *Spirogyra* sp., another a small number of *Chara* oospores.

Seeds. Seeds of *Scirpus acutus* were present in all but four of the 85 stomachs examined and was the item of first importance in volumetric percentage. Some stomachs contained a small quantity, others up to 500 or more, and in 23, represented 50% or over of the food eaten. Seeds of *Potamogeton pectinatus* occurring 57 times, and in four stomachs representing 50% or more of the total contents, was the species of next importance. Other seeds were identified the number of times indicated, viz.: *Potamogeton foliosus*, 1; *P. pusillus*, 4; *P. heterophyllus*, 6; *Myriophyllum spicatum*, 3; *Carex* sp., 2; *Sparganium* sp., 1; *Rumex* sp., 1; *Polygonum amphibium*, or *Muhlenbergii*, 8; *P. aviculare*, 2; *P. convolvulus*, 1; *Ceratophyllum demersum*, 2.

Grain. Grain was not present in any specimens taken prior to 1942. In that year seven of the 47 specimens collected had eaten wheat, undoubtedly

TABLE XIV

CONTINUED ASSOCIATION OF MALLARDS

Sex	Station banded	Date banded	Locality where recovered	Date recovered	
♀ ♀	3	Jan. 29, 1932	Station 3	Dec. 3, 1933	Retrapped
♂ ♂	4	Nov. 10, 1932	Station 4	Nov. 7, 1933	Retrapped
♀ ♀	2	Nov. 15, 1932	Pitt Lake, B.C.	Oct. 22, Nov. 12, 1933	Shot
♂ ♀	3	Dec. 18, 1932	Station 3	Dec. 3, 1933	Retrapped
♂ ♀	2	Feb. 12, 1933	Pitt River, B.C.	Oct. 15, Nov. 11, 1933	Shot
♂ ♀	2	Mar. 9, 1933	Pitt River, B.C.	Oct. 22, Oct. 29, 1933	Shot
♂ ♀	1	Nov. 8, 1933	Sumas, B.C.	Dec. 1, Dec. 10, 1934	Shot
♂ ♀	1	Nov. 23, 1933	Nicomen Island, B.C.	Oct. 22, Nov. 18, 1934	Shot
♂ ♀	2	Feb. 12, 1934	Ft. Vermilion, Alta.	♂ May 14, ♀ "spring", 1934	Shot
♂ ♂	1	Mar. 8, 1934	Pitt River, B.C.	Nov. 8, Dec. 2, 1934	Shot
♀ ♀	2	Mar. 28, 1934	Pitt Lake, B.C.	Nov. 24, 1934	Shot
♀ ♀	1	Nov. 24, 1935	Harrison River, B.C.	Nov. 29, 1936	Shot
♂ ♀	1	April 8, 1936	Everett, Wash.	Nov. 10, 1940	Shot
♂ ♀	1	Dec. 2, 1938	Ladner, B.C.	Oct. 21, Nov. 17, 1939	Shot
♂ ♀	1	Dec. 3, 1938	Station 1	♂ Jan. 1, 1940, ♀ Dec. 10, 1939	Retrapped
♂ ♂	1	Dec. 7, 1938	Station 1	Dec. 5, 1939, Dec. 24, 1939	Retrapped
♂ ♀	1	Dec. 12, 1938	Station 1	♂ Nov. 7, 1939, ♀ Dec. 7, 1939	Retrapped
♂ ♀	1	Dec. 19, 1938	Station 1	♂ Dec. 14, 1939, ♀ Dec. 22, 1939	Retrapped
♂ ♀	1	Dec. 22, 1938	Station 1	♂ Dec. 30, 1939, ♀ Nov. 15, 1939	Retrapped
♂ ♀	1	Feb. 19, 1939	Station 1	Dec. 13, 1939	Retrapped
♂ ♀	1	Feb. 20, 1939	Station 1	Dec. 23, 1939	Retrapped
♂ ♀	1	Feb. 22, 1939	Station 1	♂ Dec. 17, ♀ Dec. 30, 1939	Retrapped
♂ ♂	1	Feb. 26, 1939	Station 1	Nov. 18, 1939	Retrapped
♂ ♂	1	Mar. 8, 1939	Station 1	Jan. 2, Jan. 3, 1940	Retrapped
♂ ♀	1	April 13, 1939	Station 1	♂ Nov. 19, ♀ Dec. 30, 1939	Retrapped
♂ ♀	1	Nov. 14, 1939	Matsqui, B.C.	♂ Nov. 6, ♀ Nov. 8, 1940	Shot
♂ ♀	1	Nov. 15, 1939	Chilliwack, B.C.	♂ Nov. 13, ♀ Nov. 16, 1940	Shot
♂ ♀	1	Dec. 9, 1939	Chilliwack, B.C.	♂ Nov. 24, ♀ Nov. 26, 1940	Shot
♂ ♀	1	Dec. 10, 1939	Station 1	♂ Mar. 21, 23, ♀ Mar. 3, 31, 1940	Retrapped
♂ ♀	1	Dec. 17, 1939	Station 1	♂ Mar. 13, ♀ Mar. 15, 1940	Retrapped
♂ ♂	1	Dec. 23, 1939	Station 1	Feb. 4, Mar. 19, 1940	Retrapped
♂ ♀	1	Dec. 25, 1939	Station 1	Nov. 24, 1940	Retrapped

taken from adjacent stubble fields. In three stomachs wheat kernels composed 99 or 100%, in two others 75%, and in another five per cent of the total contents. Another contained an equal quantity of oat and wheat husks. The amount varied from a few kernels to 75 cc.

Miscellaneous vegetation. In this category are included six occurrences of *Potamogeton* foliage, two of *Ceratophyllum demersum*, three of *Myriophyllum spicatum*, one of grass, and 23 of comminuted vegetable matter not further identified. Altogether this vegetation represented a total percentage volume of 12.59%.

Babine Lake, January, 1.

Salmon eggs. The single specimen contained 316 whole eggs of sockeye salmon, *Oncorhynchus nerka*, of which 120 were eyed, and a quantity of broken salmon egg-cases. Salmon egg material represented 95% of the food in a full stomach.

Aquatic insects. The remaining five per cent of food in this stomach was composed of 70± caddis larvae, three crane fly larvae, and three coleopterous larvae.

Summary

At Henderson Lake and Quinsome Lake salmon eggs formed the chief food of 11 specimens; salmon flesh, an unidentified fish, caddis, and miscellaneous vegetation also had been eaten. The food eaten on other freshwater areas on Vancouver Island was composed of 62.4% vegetable matter, including seeds, grain, and plant material, 33.6% aquatic insects, and 4% mollusca. In the Lower Fraser Valley weed seeds and seeds of aquatic plants were of first and various grasses of second importance in the food eaten by 90 mallards. Specimens from Pitt Meadows had eaten seeds, largely *Carex*, exclusively. Mallards taken in a salt water habitat had eaten algae, small crustaceans, and molluscs.

In the Cariboo region vegetable matter constituted over 72% and various small animals, of which molluscs were first in importance, made up the remainder. At Swan Lake seeds of *Scirpus acutus* provided the largest single item in the 57% total of vegetation eaten by 85 mallards. The remainder consisted of miscellaneous seeds, foliage, and grain. Odonata and corixids with a combined percentage volume of over 41% were of next importance. Mollusca are a minor item of diet on this lake. Mallards from other lakes in the Okanagan had consumed vegetable matter, including *Chara* but largely composed of the seeds of aquatic plants, to the extent of over 80%, the balance being aquatic insects and small aquatic animals. One specimen from Babine Lake had eaten over 120 sockeye salmon eggs.

Economic Status

From an economic standpoint the mallard is the most valuable of the duck species in British Columbia. Except on parts of the coast region where at certain times of the year a diet of salmon eggs and salmon flesh renders the flesh unpalatable it is the duck most highly prized by the hunter both for its game qualities and for the table.

In connection with its pursuit is involved a large number of industries and services including among other things the manufacture, distribution, and sale of motor cars, guns, ammunition, boats, and hunting equipment of various kinds; the transport of hunters and their equipment by rail, motor bus, and by other means of travel; the hotel and cabin-camp business, and the business of guiding.

Large sums of money are invested in hunters' equipment, in land used partly or exclusively for hunting and in numerous other enterprises each deriving part of its revenue from duck hunting, which in large degree means the hunting of mallards. The investment value of the latter cannot be assessed but the value of the investment in equipment may be estimated on the following basis. In 1941 a total of 39,932 hunting licenses were issued

to resident hunters in British Columbia. Assuming for the purpose of this discussion that each licensee is a duck hunter, actual or potential, and has spent a minimum sum of $50 on equipment the investment would total $1,996,600.

The sum distributed annually by duck hunters amongst various industries and services fluctuates considerably from year to year. On the basis of the license sales for 1941, and assuming that each of the licensees spent $25 in connection with his avocation, this item would total $998,300 for that year without the addition of the sum spent by non-resident hunters of whom 511 were licensed. The total of licensed hunters resident and non-resident was 40,443. From this source the province derived a revenue of $140,507.

TABLE XV

MALLARDS BANDED IN THE UNITED STATES, AND RECOVERED IN BRITISH COLUMBIA

Banding station	Date banded	Locality where recovered	Date recovered
Sequim, Wash.	Jan. 3, 1926	Sumas, B.C.	Dec. 9, 1926
Oak Harbour, Wash.	Oct. 8, 1929	Trapp Lake, B.C.	Oct. 13, 1937
Sauvies Island, Oregon	Sept. 5, 1928	Mission City, B.C.	Oct. 15, 1928
Pilot Rock, Oregon	Jan. 25, 1934	Chilako River, B.C.	April 15, 1938
Stanfield, Oregon	Jan. 14, 1935	Scuitto Lake, B.C.	Sept. 15, 1936
Stanfield, Oregon	Jan. 17, 1935	Torphy River, B.C.	Oct. 19, 1938
Stanfield, Oregon	Jan. 17, 1935	Wynndel, B.C.	Nov., 1938
Stanfield, Oregon	Feb. 13, 1935	Kamloops, B.C.	Sept. 24, 1939
Burns, Oregon	Oct. 23, 1933	Nation River, B.C.	Aug. 28, 1938
Burns, Oregon	Nov. 25, 1935	Fort McLeod, B.C.	Sept. 17, 1938
Burns, Oregon	Nov. 3, 1936	Nechako River, B.C.	Oct. 2, 1939
Burns, Oregon	Nov. 3, 1936	Kamloops, B.C.	Sept. 26, 1937
Burns, Oregon	Oct. 21, 1938	Nechako River, B.C.	Sept. 21, 1939
Moiese, Mont.	Oct. 31, 1927	Sirdar, B.C.	Nov. 25, 1929
Moiese, Mont.	Nov. 7, 1927	Wynndel, B.C.	Oct. 25, 1928
Moiese, Mont.	Nov. 13, 1927	Kootenay Flats, B.C.	Oct. 31, 1929
Moiese, Mont.	Nov. 25, 1927	Golden, B.C.	Autumn, 1929
Moiese, Mont.	Sept. 24, 1928	Wynndel, B.C.	Oct. 19, 1929
Moiese, Mont.	Nov. 5, 1929	Vernon, B.C.	Sept. 20, 1930
Nampa, Idaho	Oct. 29, 1936	Fort St. John, B.C.	Oct. 13, 1937
Portage des Sioux, Mo.	Mar. 2, 1925	Burnaby Lake, B.C.	Dec. 29, 1927

The above represents value in terms of invested capital and of revenue both direct and indirect. There is also a value in terms of food. Figures of the annual mallard kill are lacking but on the basis of only one mallard allotted to each two hunters the number shot would be 20,221. Each mallard represents at least one dollar of value in terms of food.

The feeding habits of the mallard have been the cause of an apparent conflict of interests between hunters on the one hand and farmers and fishing interests on the other. As pointed out earlier some mallard populations feed exclusively on salmon eggs and salmon flesh during several of the autumn and winter months. Whether or not consumption of the former is responsible for a reduction in the salmon population that might not otherwise take place has not, and probably never can be determined (4).

In British Columbia the consumption of grain by mallards has not reached the proportion that it has in the Prairie Provinces. In British Columbia grain crops usually are harvested prior to the arrival of any large mallard migration. Consequently most of the feeding is done on the stubble and involves slight if any loss to the farmer. No mallards feeding in standing grain or on grain in stook have been observed.

A habit of greater concern than feeding in the grain fields is that which leads to the destruction of young forage plants in the lower Fraser Valley. When fields are partly flooded, mallards, usually in company with other pond ducks, sometimes concentrate on a small area and pull up or otherwise destroy so many young clover plants or grasses that a bare patch in the field results.

The consumption of salmon eggs by mallards, or by other ducks, is a natural process that probably has persisted through countless generations of both ducks and salmon and quite conceivably is of benefit to the salmon as well as to the ducks. The subject is highly controversial but it seems reasonable to conclude that the consumption by various animals of dead salmon and surplus salmon eggs, is necessary to a healthy biota on any salmon stream. The relation between mallards and agriculture is in quite a different category and so far as known benefits only the ducks. It would be idle to attempt comparative valuations between the economic value of the mallard and of the salmon or of the mallard and the limited agricultural interests with which it is involved. It seems advisable, however, to stress the necessity for careful consideration of the economic value of the mallard when control of its numbers is contemplated on the grounds that it reduces agricultural crops or the supply of salmon.

Acknowledgments

Grateful acknowledgment is made to Dr. R. E. Foerster, Director, Pacific Biological Station, and the Fisheries Research Board of Canada for the use of laboratory facilities; to Dr. Foerster and Dr. A. L. Pritchard for helpful criticism during the preparation of this paper; to Dr. I. McTaggart Cowan, Mr. A. L. Peake, Mr. Allan Lyon, Mr. J. Sugden, and Mr. F. M. Shillaker for the use of unpublished records and to the British Columbia Game Commission for permitting use of the banding data in its files.

References

1. COWAN, I. McT. British Columbia Provincial Museum, Occasional Papers, No. 1. 1939.
2. MUNRO, J. A. Can. Field Nat. 37 : 70-75. 1923.
3. MUNRO, J. A. Can. Field Nat. 37 : 81-83, 107-116. 1923.
4. MUNRO, J. A. Can. J. Research, D, 17 : 178-186. 1939.
5. MUNRO, J. A. and CLEMENS, W. A. Biol. Board Can. Bull. No. 55. 1937.
6. STANWELL-FLETCHER, J. F. and T. C. British Columbia Provincial Museum, Occasional Papers, No. 4. 1943.
7. SWARTH, H. S. Proc. Calif. Acad. Sci. (Ser. 4) 23(2) : 35-58. 1936.

THE VITAMIN B_1 CONTENT OF CANNED PORK[1]

By E. J. Reedman[2] and Leonard Buckby[3]

Abstract

Canned pork of the type known as spiced luncheon meat was found to contain an appreciable amount of vitamin B_1 after the complete canning process. There were, however, losses of the original vitamin B_1 in the raw meat during the retorting process used for cooking and commercially sterilizing the product. These losses were 55.3%, 55.6%, and 41.9% of the original vitamin in three series of packs examined. It was possible to fortify 6-lb. export packs of canned pork with synthetic thiamin to a level equal to, or above, that of the natural vitamin in raw meat. The destruction of synthetic added vitamin was no greater than that of natural vitamin under the conditions of this study.

Introduction

Raw lean pork muscle is known to contain an appreciable amount of vitamin B_1, and has been shown to be a richer source of this food factor than beef, veal, or poultry meat (1, 3, 5). Pork, in various forms, may thus constitute an important source of vitamin B_1 in human nutrition. However, the true value of a food must be assessed after preparation for consumption. Waisman and Elvehjem (5) found considerable destruction by the frying, broiling, or stewing of meats; losses approaching 50% were fairly common. A large amount of pork is now being canned for domestic consumption and for export. A study was made of the destruction of vitamin B_1 during the cooking and commercial sterilization of canned pork in order to establish data on the thiamin content of canned product, and to ascertain the destruction of natural and added synthetic vitamin B_1; but no attempt was made to survey the vitamin B_1 content of commercial canned pork.

Material

The canned product studied was of the type known as spiced luncheon meat, packed in 6-lb. rectangular cans. A few analyses of raw pork meat and of canned luncheon meat from domestic 12-oz. packs are reported in Table I for purposes of comparison.

Canned luncheon meat is marketed as Canadian Spiced Pork and Canadian Spiced Ham, and is manufactured from lean pork trimmings and lean sow ham, respectively. The raw meat is trimmed to 20% or less fat, but varies somewhat in fat content. The raw meat is ground, vacuum mixed with curing salts and sugar, and cured at 38° F. for 48 hr. (or by a similar process). After curing, the product is again vacuum mixed, is stuffed raw into the cans,

[1] *Manuscript received May 7, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Published as paper No. 93 of the Canadian Committee on Food Preservation, and as N.R.C. No. 1132.

[2] *Biochemist.*

[3] *Laboratory Assistant.*

which are then vacuum closed and retorted to cook and commercially sterilize the meat. The only major source of destruction of vitamin B_1 is this heating process.

TABLE I

MOISTURE AND VITAMIN B_1 CONTENT OF SAMPLES

Description of sample	No. of lots or cans analysed	Moisture, %		Vitamin B_1, μgm./gm. (moisture-free basis)	
		Range	Mean	Range	Mean
1. Raw lean chop pork, Series I	3	66.5–70.1	69.2	31.5–63.8	52.0
2. Raw lean chop pork, Series II	3	64.3–71.7	68.7	32.7–48.0	41.9
3. Domestic 12-oz. cans, Commercial Pack *A* (pork)	3	59.1–61.8	60.0	11.5–13.5	12.6
4. Domestic 12-oz. cans, Commercial Pack *B* (pork)	3	55.4–58.9	57.3	8.1–10.7	9.6
5. Domestic 12-oz. cans, Commercial Pack *C* (pork)	3	57.6–58.2	58.0	9.8–10.5	10.1
6. Domestic 12-oz. cans, Commercial Pack *D* (ham)	3	56.7–57.7	57.1	8.4–11.4	9.6
7. Export 6-lb. pack, canned raw pork before retorting, Series I, II, III	15	63.1–66.1	64.8	18.3–24.6	20.7
8. Export 6-lb. pack, canned pork after retorting, Series I, II, III	15	62.6–65.7	64.2	7.5–12.6	9.8

Preliminary studies showed that there was little difference in the initial vitamin B_1 content of canned pork and ham of this type, and that there was a similar destruction of vitamin B_1 during processing; both findings are in agreement with results from other laboratories (3, 5). The investigation was therefore confined to canned pork as being representative of canned spiced luncheon meat.

Methods of Analysis

The contents of each lot or can of meat were treated as a separate entity; vitamin B_1 and moisture determinations were made jointly on the meat from each can. The lots of raw chop and loin meat were first ground through a 3/16 in. plate, and then mixed thoroughly before sampling. The raw meat from each can was mixed on a double-arm mixing device of standard design whereas retorted or cooked product was first ground and the total contents of the can then mixed before samples were taken.

Moisture determinations were made on samples placed in an air-oven at 98° C. for 24 hr.

Vitamin B_1 determinations were made by a modification of the chemical method of Jansen (2), involving the measurement of extracted thiamin as thiochrome in a photoelectric fluorometer. The sample, in each case, was incubated with pepsin and takadiastase to liberate the combined form of thiamin. It was found advantageous to buffer the extract at pH 2.0 for

the pepsin incubation (Buffer No. 1)[1], and at pH 4.5 for the takadiastase incubation (Buffer No. 2)[2].

Details of the method follow: each sample consisted of 100 gm. of meat, which was mixed with 100 ml. of distilled water on a Waring Blender to give a material of homogeneous consistency; the sample taken for vitamin B_1 analysis consisted of 5 to 10 gm. of blended material, which was accurately weighed into a 250 ml. centrifuge bottle. To the sample in the centrifuge bottle was first added 50 ml. of 0.2% pepsin in 0.33% hydrochloric acid; the pH was adjusted to 2.0 using 0.04% Thymol Blue as an external indicator, 2 ml. of Buffer No. 1 was added, and the sample was then incubated and extracted for one hour. Incubation and extraction were combined by turning the stoppered centrifuge bottle end over end at a speed of 29 r.p.m. in a constant temperature oven maintained at 37° C. The centrifuge bottle was removed from the extraction apparatus, the pH was adjusted to 4.5 using Brom Cresol Green as an external indicator, 2 ml. of Buffer No. 2 was added, and the sample further incubated and extracted for one hour at 37° C. This method was found to give maximum values for free and combined vitamin B_1 with the meat samples assayed; longer times of incubation and extraction gave no higher results. After extraction the sample was filtered to give a clear extract for the determination of thiamin. A standard procedure, using 1 or 2 ml. of this extract, made possible the conversion of thiamin to thiochrome (4); all measurements of fluorescence were made with a photoelectric fluorometer of commercial design.

Experimental

To ascertain the precision of the method of analysis and the sampling error, duplicate analyses were carried out on two 100-gm. samples drawn from each pack of a series of seven 6-lb. cans of raw meat and three 6-lb. cans of cooked

TABLE II

ANALYSIS OF VARIANCE OF SAMPLING DIFFERENCES IN VITAMIN B_1 DETERMINATIONS

Source of variance	Degrees of freedom	Mean square
Between cans	7	20,852
Within cans	10	16,910
Duplicate error	20	11,264

meat. A statistical analysis of the results showed that the degree of variation between duplicate packs and between samples from the same pack was of the same order of magnitude, and was not significant in either case (Table II).

[1] *Buffer No. 1 : 10 ml. N hydrochloric acid and 50 ml. N potassium chloride in 1. l. distilled water.*

[2] *Buffer No. 2 : 500 ml. M potassium acid phthalate and 9.8 ml. N sodium hydroxide in 1 l. distilled water.*

It was therefore considered that a single 100-gm. sample might be used as representative of lots or cans of meat by the method of sampling used.

The samples of raw chop meat, and the domestic 12-oz. cans were purchased in retail stores. The export 6-lb. packs were obtained from a commercial establishment, and were shipped to the laboratory as canned meat that had been completely packed but not cooked or commercially sterilized, i.e. raw canned meat. Three series, each consisting of 15 cans chosen as representative of three separate batches at the canning establishment, were obtained. The series were divided at random, each into three sets of five cans. One set of five cans from each series was analysed to represent raw meat as received.

One set of five cans from each series was retorted for 200 min. at a retort temperature of 230° F., and subsequently assayed for vitamin B_1. This process is the one most commonly used in industry for the cooking and commercial sterilization of canned spiced luncheon meat in 6-lb. containers. This heat treatment is equivalent to autoclaving the product within the cans, and might be expected to cause considerable destruction of vitamin B_1. Temperature gradations within the pack followed the usual course. At the end of 200 min. at 230° F., the centres of the cans had attained 222° F. Cold water was then run through the retort until the centres of the cans reached 100° F., a process that took 75 min. These time–temperature conditions approximate commercial practice.

Five cans from each of the three series were taken for a study of the destruction of synthetic vitamin B_1 added as thiamin hydrochloride crystals. Each original can was opened and the contents emptied and mixed by means of a mechanical double-arm device. Vitamin B_1 was added as synthetic crystals dissolved in 100 ml. of distilled water to the total contents of each pack. This addition was mixed thoroughly with the meat, and the product repacked and vacuum sealed in new cans. The repacked cans were then retorted for 200 min. at a retort temperature of 230° F. and cooled in running water. Synthetic vitamin B_1 was added at levels of 20 mg., 100 mg., and 200 mg. per 6-lb. pack, to cans that were distributed through the three series so that all levels of original, naturally occurring vitamin B_1 were represented.

Discussion of Results

Table I summarizes the analytical data obtained on raw and canned pork meat. Since raw chop meat samples (1 and 2) were different from the cuts used in canning, and were trimmed entirely free of fat and not mixed with any other ingredients, the analyses given cannot be compared directly with the amount of vitamin B_1 in canned product. They do, however, agree with published data on the thiamin content of raw lean pork (1, 3, 5). Little difference is shown between the vitamin B_1 values of the domestic 12-oz. packs of pork and ham and the export 6-lb. packs analysed; therefore both may be considered a good source of this factor.

The apparent destruction of vitamin B_1 during the retorting process for the 6-lb. packs is shown by Table III. The analyses given are values for the

amount of thiamin in meat from the cans of non-retorted and retorted product. The percentage destruction was calculated from these data, but it was not possible to obtain analyses on the product from the same can before and after retorting, since this would have involved changing the product by mixing and repacking. Previous experiments demonstrated that mixing and repacking, besides causing aeration of the meat, always resulted in excessive liquid rendering during subsequent retorting.

TABLE III

VITAMIN B_1 REMAINING IN CANNED SPICED PORK AFTER COMMERCIAL STERILIZATION

Description of sample	Vitamin B_1, μgm./gm. (moisture-free basis)			
	Series I	Series II	Series III	Series I, II, III
1. Average vitamin B_1 content of 6-lb. packs before retorting	19.2 (18.3–20.0)	23.6 (20.6–24.6)	20.3 (18.9–22.1)	20.7 (18.3–24.6)
2. Average vitamin B_1 content of 6-lb. packs after retorting	8.6 (7.5–10.8)	10.5 (8.7–11.7)	11.8 (11.1–12.6)	9.8 (7.5–12.6)
3. Calculated loss of vitamin B_1 during the retorting process, %	55.3	55.6	41.9	52.7

In the product to which synthetic thiamin was added, it was necessary to repack. The results given in Table IV are, therefore, for packs that are not identical with commercial product. However, since the effect of aeration and rendering would be to increase the destruction of vitamin B_1, the conditions of processing are no less severe for repacked cans. If synthetic additions

TABLE IV

VITAMIN B_1 IN CANNED PORK (MOISTURE-FREE BASIS) AFTER ADDITION OF SYNTHETIC VITAMIN B_1

Description of sample	Vitamin B_1, μgm./gm.		Indicated total loss of vitamin B_1, %
	Before retorting	After retorting	
1. Export 6-lb. packs as received	20.7 (18.3–24.6)	9.8 (7.5–12.6)	52.7
2. Export 6-lb. packs with addition of synthetic thiamin at a level of 20.5 μgm./gm. (20 mgm./pack)	41.2* (38.8–45.1)	26.7 (25.4–29.6)	35.2
3. Export 6-lb. packs with addition of synthetic thiamin at a level of 102.8 μgm./gm. (100 mgm./pack)	123.5* (121.1–127.4)	72.4 (68.4–76.6)	42.4
4. Export 6-lb. packs with addition of synthetic thiamin at a level of 205.6 μgm./gm. (200 mgm./pack)	226.3* (223.9–230.2)	136.4 (130.7–141.2)	39.8

* *Calculated values.*

were to be made in commercial practice, the appropriate stage would be during the mixing of the ingredients for the canned luncheon meat, and hence addition would result in no change of processing or appearance of the final commodity. The results given in Table IV indicate that an appreciable amount of synthetic thiamin was retained after the processing procedure used.

References

1. CHRISTENSEN, F. W., LATZKE, E., and HOPPER, T. H. J. Agr. Research, 53 : 415-432. 1936.
2. JANSEN, B. C. P. Rec. trav. chim. 55 : 1046-1052. 1936.
3. MICKELSEN, O., WAISMAN, H. A., and ELVEHJEM, C A. J. Nutrition, 17 : 269-280. 1939.
4. REEDMAN, E. J. and YOUNG, G. A. Can. J. Research, C, 21 : 145-150. 1943.
5. WAISMAN, H. A. and ELVEHJEM, C. A. The vitamin content of meat. Burgess Publishing Co., Minneapolis. 1941.

DRIED WHOLE EGG POWDER

V. DEFINITION AND PROPERTIES OF LOW GRADE EGG POWDERS[1]

By M. W. Thistle[2], Margaret Reid[2], and N. E. Gibbons[3]

Abstract

The point at which 50% of tasters regarded dried whole egg preparations as unsuitable for human consumption coincided with a rating of 2.7 on a scale ranging from 10 for excellent, fresh egg to 0 for repulsive material. The protein fraction of these low grade samples had deteriorated badly, as shown by fluorescence measurements. The fat fraction showed no evidence of peroxide oxygen formation.

Introduction

Hitherto, information with respect to the level of quality at which trained tasters consider dried whole egg powder to be unsuitable for human consumption has been rather meagre. It was observed from time to time that taster scores were more variable on low than on high quality powders, and therefore, in order to operate at these low quality levels, highly trained tasters were required. The presence of an experienced taste panel in these laboratories made possible the present investigation.

Materials and Methods

Ten samples were obtained which, on the basis of their storage history, could be expected to receive scores between 5 and 0 on the taste scale used (2, 3). Five of these samples consisted of powders produced in 1939 and 1940, and since stored in cardboard containers at room temperature; two powders had received harsh storage treatment (47.8° C. for five weeks); three powders had been stored at 36.7° C. for three months, and subsequently for a year at room temperature in cardboard containers.

The method of preparing the samples has been described (2). The scoring system ranged from 10 for excellent, fresh egg to 0 for repulsive material. The taste panel consisted of four tasters selected from the larger panel used in previous work (2, 3): this selected panel had 14 months' experience in quality control work prior to the present investigation. The 10 samples were scored by the panel described, with each taster also noting whether he considered the samples edible or inedible. The whole procedure was repeated a day later, with the samples in different order. Unfortunately one of the tasters revolted, so that most samples received seven judgments rather than eight; however, all data obtained were used in computing the results.

[1] *Manuscript received May 8, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as paper No. 94 of the Canadian Committee on Food Preservation, and as N.R.C. No. 1133.

[2] *Biochemist, Food Investigations.*

[3] *Bacteriologist, Food Investigations.*

Deterioration of the protein fraction was assessed by fluorescence values (1, 2, 3); and deterioration of the fat fraction was assessed by peroxide oxygen determinations (4).

Results

The results are shown in Table I. Repeating the experiment provided a basis for estimating panel error; the standard error for any one sample in this instance was 0.65 palatability units, as compared with a standard error of 0.28 units at the higher quality levels. This confirms the previous observation that taster scores were more variable on poor than on good quality powders.

TABLE I

RELATION OF TASTER SCORE TO "INEDIBILITY"

Description of sample	Average score, 1st day	Average score, 2nd day	Final score	In-edibility ratings, %
Three years old	4.0	4.0	4.0	0
Three years old	3.3	3.8	3.6	14
Four years old	2.7	3.5	3.1	57
Three years old, dried from Grade *C* eggs	2.3	3.5	3.0	57
Stored at 47.8° C. for five weeks, 2.3% moisture content	2.8	3.1	2.9	12
Canned in CO_2, stored at 36.7° C. for three months, one year since treatment	3.7	2.0	2.7	43
Canned in air, stored at 36.7° C. for three months, one year since treatment	2.3	2.0	2.1	57
Open pack, stored at 36.7° C. for three months, one year since treatment	2.3	2.0	2.1	100
Stored at 47.8° C. for five weeks, 4.5% moisture content	2.5	1.5	2.0	62
Dust-collector powder, three years old	0.7	2.0	1.4	100

On the basis of the seven (or eight) judgments available for each sample, 100% considered the sample rated 4.0 as edible, while 100% considered the sample rated 1.4 as inedible. The remaining eight samples received intermediate taster scores, and with one exception, intermediate edibility ratings.

A correlation of $-.82^{**}$ was observed between "percentage inedibility" ratings and taster score. This relationship is shown graphically in Fig. 1. The point at which 50% of the tasters considered a sample to be unsuitable for human consumption coincides with a taster score of 2.7 (computed, $y = -34x + 142$).

Fluorescence measurements on these particular powders were unusually difficult to make. Certain of the defatted powders did not disperse properly in the protein solvent, but formed small balls of dry powder, the surfaces only coming in contact with the solvent. This was true of powders that had been held in cardboard containers at room temperature for a year or longer; the remaining powders behaved normally. However, the fluorescence values

** *Exceeds the 1% level of statistical significance.*

obtained all showed marked deterioration in the protein fraction; the average over the 10 samples was 130 photofluorometer units, corresponding to the average taster score of 2.69.

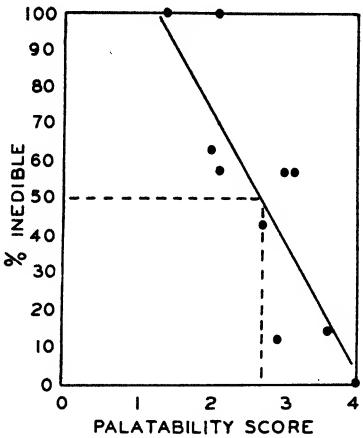


FIG. 1. *Relation of "percentage inedibility" ratings to taster score.*

It has previously been noted that considerable deterioration occurred in flavour quality without any change being detected by peroxide oxygen measurements (2). Badly deteriorated as these powders were, no evidence could be obtained of any trace of peroxide oxygen formation. Dried egg powder therefore appears to differ somewhat from other high-fat foods, as far as the behaviour of the fat fraction is concerned. This point is receiving some attention.

References

1. PEARCE, J. A. Can. J. Research, D, 21 : 98-107. 1943.
2. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20 : 276-282. 1942.
3. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
4. WHITE, W. H. Can. J. Research, D, 19 : 278-293. 1941.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. SEPTEMBER, 1943 NUMBER 9

DRIED WHOLE EGG POWDER

VI. EFFECT OF STORAGE TEMPERATURE AND GAS PACKING ON KEEPING QUALITY[1]

BY W. HAROLD WHITE[2], M. W. THISTLE[2], AND MARGARET REID[2]

Abstract

Dried whole egg powders were obtained from three different manufacturers and stored at temperatures ranging from 7.1° to 32.1° C. for periods up to six months. Quality was assessed by determination of fluorescence and potassium chloride values. At 23.8° C. the rate of deterioration was comparatively rapid; at 32.1° C. it was markedly so. To maintain quality during storage and transport dried egg should be stored at a temperature of 15.6° C. or lower.

The effect on keeping quality of packing in nitrogen, carbon dioxide, *in vacuo*, or in the form of compressed tablets was studied. Carbon dioxide alone had beneficial effect.

Introduction

In a recent investigation it was shown that the quality of dried whole egg powder was materially affected by the rate at which the powder was cooled after drying (3). The observed thermal instability of egg powder indicated that a study of suitable conditions for storage and transport was necessary. The present paper deals with the effect of temperature, gas packing, and compression on the keeping quality of dried whole egg powder during storage.

Materials and Procedure

Storage Temperature

Egg powder was obtained from three representative Canadian egg drying establishments. The powder from Plant I was prepared from a mixture of 95% Grade A and 5% Grade B fresh eggs dried in a box-type drier at inlet and outlet temperatures of 150° and 58° C. (302° and 137° F.) respectively; that of Plant II from a 1:1 mixture of Grade A and B fresh eggs dried in a modified box-type drier at inlet and outlet temperatures of 108° and 59° C. (227° and 138° F.) respectively; and that of Plant III from Grade A fresh eggs at inlet and outlet temperatures of 110° and 51° C. (230° and 123° F.) respectively.

[1] *Manuscript received June 16, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. A portion of the material was selected from a thesis presented by one of us (W.H.W.) to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as paper No. 96 of the Canadian Committee on Food Preservation and as N.R.C. No. 1140.

[2] *Biochemist, Food Investigations.*

Samples of powder (100 gm.) from each of the three plants were placed in cardboard containers with metal ends and stored at temperatures of 7.1°, 15.6°, 23.8°, and 32.1° C. (45°, 60°, 75°, and 90° F.). The use of this type of container for storage was prompted by the present extensive application of "ersatz" containers in commercial practice. Samples were removed for analysis after storage for one, two, four, and six months at 7.1°, 15.6°, and 23.8° C. and at semimonthly intervals for a total period of three and one-half months at 32.1° C. Changes in quality were assessed by determination of the fluorescence and potassium chloride values (1, 2).

Gas Packing

The dried whole egg powder used in the gas-packing studies was similar to that secured from Plant II for the storage experiment. Five different methods of packing were studied, namely, in air, carbon dioxide, or nitrogen; under vacuum; and compressed in the form of a 100-gm. tablet. Samples to be packed in gas or *in vacuo* were placed in glass flasks, thoroughly evacuated with a Hyvac pump, flushed three times with gas where desired, and the flasks sealed off. The tablets were compressed under 800 lb. per sq. in. at room temperature. Pressure was increased rapidly to the desired level and released immediately to minimize exudation of egg oil. The tablets and air-packed material were both stored in sealed tin cans.

Duplicate samples were removed for analysis after storage for one, two, and six months at 23.8° C. (75° F.). Quality of the powders was assessed by determination of the fluorescence and potassium chloride values (1, 2).

Results

Storage Temperature

The effect of storage temperature, storage time, and source of powder on quality may be seen from the mean fluorescence and potassium chloride values given in Table I. Differences with temperature were greatest between 23.8° and 32.1° C. and least between 7.1° and 15.6° C. The changes in mean fluorescence values with time for powders stored at 7.1° to 23.8° C. were greatest between the two-, four-, and six-month periods, whereas at 32.1° C. the values increased rapidly during two and one-half months and subsequently remained essentially at the same level. Similarly the potassium chloride values showed marked decrease between two and four months at 7.1° to 23.8° C. and during the first one and one-half months in storage at 32.1° C.

The relative order of the mean fluorescence and potassium chloride values for the three plants was the same after storage as observed initially. However, the magnitudes of the differences between plants, in terms of mean fluorescence values of the powder, were slightly smaller after storage, while those in mean potassium chloride values were larger. Thus, greater changes occurred during storage in powders of low than of high fluorescence values. The opposite behaviour was observed for the potassium chloride values except

TABLE I

MEAN FLUORESCENCE AND POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDERS AFTER STORAGE FOR VARIOUS PERIODS AT 7.1° TO 32.1° C.

Temperature

Temperature, °C.	7.1	15.6	23.8	32.1
Mean fluorescence value [1, 2]	27.5	29.2	33.7	49.6
Mean KCl value [1, 2]	66.5	63.4	54.6	41.5

Storage period

Time, months	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	6.0
Mean fluorescence value[1]	28.1	–	27.3	–	26.4	–	–	–	30.5	36.3
Mean fluorescence value[2]	28.1	38.7	40.2	49.3	55.1	62.7	62.9	59.5	–	–
Mean KCl value[1]	71.4	–	69.9	–	68.2	–	–	–	54.2	53.8
Mean KCl value[2]	71.4	42.0	67.6	33.1	31.8	29.6	29.2	26.8	–	–

Plant

Plant	I	II	III
Mean fluorescence value[1]	36.0	28.8	25.6
Mean fluorescence value[2]	51.8	50.1	46.8
Mean KCl value[1]	53.2	66.4	65.0
Mean KCl value[2]	38.5	44.9	41.0

[1] *Mean values over all other conditions studied for powder stored at 7.1°, 15.6°, and 23.8° C.*
[2] *As for* [1], *but on powder stored at 32.1° C. only.*

at 32.1° C. where the differences between plants were about the same as they were initially. This point will be discussed further in a subsequent paper.

Analyses of variance were used to assess the relative importance of the factors studied. Since the sampling times employed at 32.1° C. differed from those at the lower temperatures, these data were treated separately. The source of the powder and temperature and time of storage all had highly significant effects on both the fluorescence and potassium chloride values except at 32.1° C. where the differences in potassium chloride values between plants failed to reach the level of statistical significance (Table II).

Of the differential quantities, that relating temperature with storage period was alone significant. Details of the changes in fluorescence values during storage at 7.1° to 23.8° C., and at 32.1° C. are given in Tables III and I respectively. The fluorescence values for powders stored at 7.1° and 15.6° C. were essentially unchanged after four months. In this connection it is

TABLE II

ANALYSES OF VARIANCE OF FLUORESCENCE AND POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDERS STORED FOR VARIOUS PERIODS AT 7.1° to 32.1° C.

Storage temperature, °C.	Source of variance	D.f.	Mean square	
			Fluorescence value	KCl value
7.1°, 15.6°, and 23.8° C.	Temperature	2	249.2**	917.7**
	Time	3	366.3**	1366**
	Plants	2	679.7**	1268**
	Stored vs. non-stored	1	21.97*	544.8**
	Temperature × time	6	21.02**	160.7*
	Temperature × plants	4	0.19	45.06
	Residual	20	4.27	
	Duplicate error	39	0.50	3.20
32.1° C.	Time	6	619.7**	1219**
	Plants	2	100.1*	166.3
	Stored vs. non-stored	1	3146**	6171**
	Residual	14	21.10	91.03
	Duplicate error	24	1.514	1.21

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

probable that the values for four and six months' storage at 7.1° C. are somewhat higher than would normally be obtained since the moisture content of the powders increased from five to about seven per cent in this period. It has recently been shown that moisture content has considerable effect on the keeping quality of egg powder (4). The reason for the apparent decrease in the fluorescence values after one and two months is unknown. Since the fluorescence values increased at a relatively rapid rate at 23.8° C. and markedly so at 32.1° C., it is apparent that egg powder should be held at a temperature of 15.6° C., and preferably lower, if quality is to be maintained during storage and transport.

TABLE III

FLUORESCENCE VALUES OF DRIED EGG POWDERS STORED FOR VARIOUS PERIODS AT 7.1°, 15.6°, AND 23.8° C.

Time, months	Temperature, °C.		
	7.1	15.6	23.8
1	26.0[1]	26.9[2]	28.8
2	23.7	24.5	30.9
4	29.0	29.7	33.0
6	31.3	35.5	42.2

[1] *All values as the means for three plants.*
[2] *Necessary difference to exceed 5% level of statistical significance: 2.5.*

While the potassium chloride values of egg powder decreased during storage at all temperatures, the changes were least and approximately of the same magnitude at 7.1° and 15.6° C. (Tables IV and I). This confirms the conclusions based on fluorescence values, and emphasizes the necessity for storing whole egg powder at temperatures of 15.6° C. or lower.

TABLE IV

POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDERS STORED FOR VARIOUS PERIODS AT 7.1°, 15.6°, AND 23.8° C.

Time, months	Temperature, °C.		
	7.1	15.6	23.8
1	71.9	67.1	70.8
2	71.4	70.7	62.4
4	61.0	57.9	43.6
6	61.8	58.1	41.6

[1] *All values as the means for three plants.*
[2] *Necessary difference to exceed 5% level of statistical significance: 8.0.*

Gas Packing

The effect of the various methods of packing studied on the fluorescence and potassium chloride values are given, together with analyses of variance, in Tables V and VI. Packing in carbon dioxide had definite preservative effect, as indicated by fluorescence and especially by potassium chloride values. Storage in nitrogen, under vacuum, or in the form of a compressed tablet apparently had little effect. The suitability of gas packing with carbon dioxide is being given further study.

TABLE V

EFFECT OF VARIOUS METHODS OF PACKING ON THE FLUORESCENCE VALUES OF DRIED EGG POWDER STORED AT 23.8° C.

Mean values

Storage period, months	Method of packing				
	Air	Vacuum	Carbon dioxide	Nitrogen	Tablet
1	25.5[1]	27.6	25.5	28.9	28.7
2	29.2	31.5	24.8	31.2	28.8
6	44.5	61.4	38.6	46.5	42.4

Analysis of variance

Source of variance	D.f.	Mean square
Method of packing	4	179.9**
Time	2	2299**
Method of packing × time	8	82.37**
Duplicate samples	15	5.352
Duplicate analyses	30	0.8588

[1] *Necessary difference to exceed 5% level of statistical significance: 3.5.*
** *Indicates 1% level of statistical significance.*

TABLE VI

EFFECT OF VARIOUS METHODS OF PACKING ON THE POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDER STORED AT 23.8° C.

Mean values

Storage period, months	Method of packing				
	Air	Vacuum	Carbon dioxide	Nitrogen	Tablet
1	64.3[1]	66.2	71.6	70.4	61.8
2	59.8	56.3	73.2	55.8	57.8
6	39.1	37.8	72.2	51.6	40.7

Analysis of variance

Source of variance	D.f.	Mean square
Method of packing	4	783.7**
Time	2	1786**
Method of packing × time	8	165.0
Duplicate samples	15	8.714
Duplicate analyses	30	1.844

[1] *Necessary difference to exceed 5% level of statistical significance: 4.5.*

** *Indicates 1% level of statistical significance.*

Acknowledgments

The authors wish to express their thanks to Mr. T. A. Steeves, Refrigeration Engineer, for his assistance in establishing and maintaining the storage conditions, and to Mr. L. Moore, Laboratory Assistant, for his technical assistance.

References

1. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20: 276-282. 1942.
2. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
3. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 194-202. 1943.
4. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 211-222. 1943.

THE DEVELOPMENT OF THE WILD TYPE AND BAR EYES OF *DROSOPHILA MELANOGASTER*[1]

BY ARTHUR G. STEINBERG[2]

Abstract

The histological development of the eyes of Bar and wild type *Drosophila melanogaster* during the larval and pupal stages is described. The onset of morphological differentiation of the ommatidia of both Bar and wild type occurs between 48 and 72 hr. after hatching from the egg (temperature = 25° ± 0.2°C.). At all stages the developing ommatidia of Bar and wild type are the same. At no time was evidence of degenerating ommatidia found in any region of the Bar eye. The data are discussed in the light of several hypotheses that have been advanced to explain the development of the Bar eye.

Introduction

When Drosophila geneticists turned to the study of the role of the gene in development, they were, and in fact still are, repeatedly handicapped by a lack of knowledge of the normal development of this organism. Before Poulson could satisfactorily study the effect of various deficiencies on the development of the embryo (16) he was compelled to do a pioneer study of the normal ontogeny of the early embryo (15). Kaliss (9) in his study of the development of the yellow achaete deficiency found it necessary to describe the normal ontogeny of the late embryo; the situation is similar with regard to studies on the wings and limbs (1, 5, and 23).

Although the eye is probably the organ most studied by those interested in developmental genetics, and many theories concerning the mode of action of at least one mutant (Bar) on its development have been elaborated, relatively little is known about its development. (This may be an important reason for the many theories elaborated to explain the action of the Bar mutation.) The few studies of the histological development of the eye that have been made have led to contradictory conclusions. A brief survey of these studies follows.

The time of onset of the histological differentation of the ommatidia has been reported by Krafka (10) and Chen (3) to be 24 hr. before pupation (temperature not given). Medvedev (12) reported that the "ommatidia" can be seen in stained whole mounts of optic disks of mature larvae but made no mention of studies of earlier stages. On the other hand Bodenstein (2) and Robertson (11) claim that the ommatidia do not begin to differentiate until at least 36 hr. after puparium formation. In contrast to the above reports Enzmann and Haskins (4) state that they observed the onset of ommatidial differentation as early as 18 hr. after egg deposition. The author (19) has already reported that he was unable to confirm Enzmann and Haskins' obser-

[1] *Manuscript received June 22, 1943.*
Contribution from the Department of Genetics, McGill University, Montreal, Que.
[2] *Lecturer.*

vations. Recently Pilkington (14) reported the results of studies of the histological development of the normal and some mutant eyes. He claimed that up to "the four day larva the optic disc (consists) of a mass of cells several layers thick but apparently homogenous." This claim is certainly not so as shown by the present author (19) and also Krafka (10). Pilkington's detailed description of the subsequent development of the eye will be reviewed below.

The resolution of these contradictions became particularly urgent to the present author when his study of the growth curves of wild type, Bar, and modified Bar eye disks (19 and 20) led him to conclude that the reduced number of facets exhibited by the Bar eye was the result of a reduction of the number of cells entering into the formation of the cephalic complex; it was also postulated that the range of facet number over which an eye is able to vary is determined by the presence of a number of cells that are labilely determined to form facets or head chitin, depending upon the action of extrinsic and intrinsic factors (19 and 20).

Preliminary histological examination of wild type eye disks showed that in the wild type the cells of the optic disk begin to organize into "clusters" of four cells each at about 72 hr. after hatching from the egg at 25° C. The "clusters" increased greatly in both number and clarity by 96 hr. after hatching (19). However, these findings could not be confirmed for Bar at that time; i.e., no organization of cells into larger units was observed.

The questions left open therefore were (*a*) when, if at all, do these "clusters" arise in the Bar eye disk and (*b*) what is the subsequent role of these "clusters" in the development of the eye?

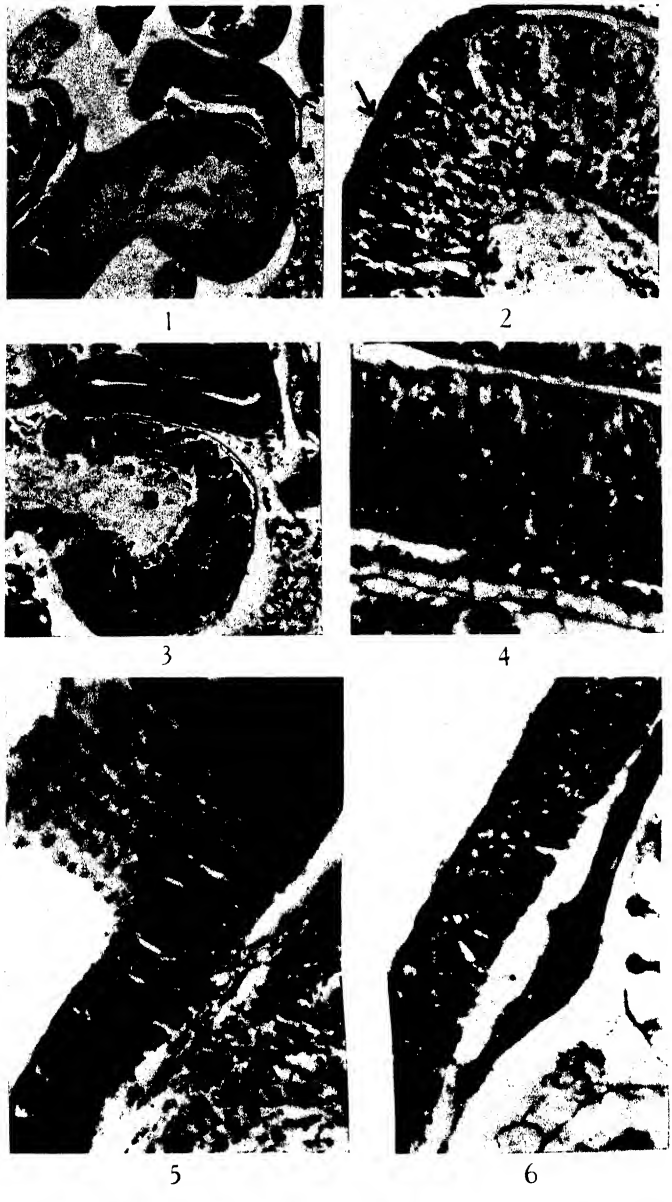
This paper is a report of studies undertaken to obtain answers to these questions. An abstract of part of the data discussed below is published in *Genetics* (21).

Material and Methods

The stocks of *Drosophila melanogaster* used for these experiments were (*a*) an inbred Florida wild type and (*b*) an inbred Bar that had been rendered isogenic with the Florida strain by repeated backcrossing. Both these stocks had been inbred for more than 100 generations by the beginning of these experiments.

All larval ages were determined from the time of hatching and were accurate to within one hour. The larvae were raised in 3-in. Petri dishes (20 to 30 larvae per dish), on a food medium consisting of 2% agar plus 12.5% of molasses in water, seeded 24 hr. before use with two drops of a heavy yeast suspension.

All pupal ages were determined from the time of puparium formation. The onset of puparium formation was taken as the time when the larva was no longer capable of moving its anterior spiracles in response to a stimulus. For the sake of simplicity of discussion, both prepupae and pupae will be referred to as pupae (true pupation occurs 12 hr. after puparium formation



All prints are contact prints. All magnifications given are initial magnifications at the ocular. B = brain; E = eye disk.

FIG. 1. *Wild type larva 96 hr. after hatching. 140×.*

FIG. 2. *High power photograph of a portion of the eye disk shown in Fig. 1. 840×. Several "goblets" may be seen. The arrow points to a particularly clear one.*

FIG. 3. *Wild type "pupa" five hours after puparium formation. 140×.*

FIG. 4. *High power photograph of a portion of the eye disk shown in Fig. 3. 840×. The arrow indicates one of five "goblets" shown.*

FIG. 5. *Section through an eye disk of a six hour wild type pupa. 600×. Because the disk was curved the ommatidial precursors were cut in cross section in one portion ("clusters" at upper left) and in longitudinal section in another ("goblets" at lower right).*

FIG. 6. *Longitudinal section through the eye of a 12 hr. wild type pupa. 840×.*

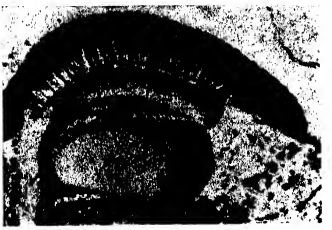
7

8

9

10

11



12

13

All prints are contact prints. All magnifications given are initial magnifications at the ocular. A = antennal disk; B = brain; E = eye disk; N = optic nerve.

FIG. 7. *Frontal section through a 10 hr. wild type pupa. 140 ×. Note the position of the optic nerve.*

FIG. 8. *Longitudinal section through the eye of an 18 hr. wild type pupa. 840×.*

FIG. 9. *Longitudinal section through the eye of a 24 hr. wild type pupa. 840×.*

FIG. 10. *Longitudinal section through the eye of a 48 hr. wild type pupa. 840×.*

FIG. 11. *Cross section through the eye of a 48 hr. wild type pupa. 840×.*

FIGS. 12 AND 13. *Longitudinal section through the eye of a 72 hr. pupa. 140× and*

at 25° C.). Throughout these experiments the temperature used was 25 ± 0.2° C.

Previous experience (19) had shown that best histological results were obtained by fixing at 50° C. for one-half to one hour in a modified Carnoy mixture consisting of glacial acetic, two parts; chloroform, three parts; and absolute alcohol, five parts. Consequently all fixations were done with this solution. Both larvae and pupae were sectioned at 10 μ and stained in Delafield's haematoxylin with eosin as a counterstain.

Data

Wild Type

Re-examination of the larval eye disks at 48, 72, and 96 hr. after hatching served to confirm the author's previously reported observations (19). There is no organization of the cells into units of a higher order before 72 hr. of larval life. The units then formed are few in number and are seen to consist of clusters of four cells each (however, see below). These "clusters" increase greatly in number by 96 hr. They can be readily seen in whole mounts lightly stained in eosin, as reported by Medvedev (12), Krafka (10) and others, including the present author. These observations in no way coincide with Pilkington's (14) diagrams of the eye disks of mature larvae (compare Figs. 2, 4, 15, and 17 of this paper and his Text Fig. 1A). As a matter of fact his diagrams of subsequent stages cannot at all be reconciled with the present author's observations. This is clearly brought out by a comparison of Pilkington's Text Fig. 1 and the photographs in Plates I and II of this paper.

Among the first slides examined of pupal stages was one of a five hour old pupa. It showed with startling clarity a series of "goblet-like" structures arranged in a very regular manner, their large diameter directed away from the optic nerve (Fig. 4). Further investigation made it clear that these "goblets" were the result of longitudinal sections through structures that, in cross section, had the appearance of the cell "clusters" described above. This is well illustrated by Fig. 5, a photograph of a section through the eye disk of a six hour old pupa. A search for these structures was made in 72- and 96-hr. larvae. They were found in larvae of both stages—many more of them in 96-hr.- than in 72-hr.-old larvae. Fig. 2 is a photograph of the "goblets" as seen in a 96 hr. old wild type larva. That these "goblet-like" structures are the precursors of the ommatidia is clearly shown by a study of later and later pupal stages (Figs. 1 through 13). The "goblets" undergo various minor changes in shape during their development. Just after eversion of the eye disks they are somewhat foreshortened and "wedge-shaped" (Fig. 6). Six hours later they are again "goblet-shaped" (Fig. 8). After another six hours they are again foreshortened and "wedge-shaped", differing from the 12 hr. stage in coming to a sharper wedge (Fig. 9). At all these stages it is possible to trace in at least some regions a single nerve fibre entering each "goblet", in favourable locations these fibres can be seen separating from the

large, common optic nerve (Figs. 6 and 9). This has been observed in larval as well as pupal stages. During the next 24 hr. the ommatidial precursors become very much foreshortened, as though compressed, and assume in longitudinal section (i.e. longitudinal section through them) an almost circular shape. The nervous connection is much clearer (Fig. 10). In addition there is now, for the first time, clear indication of the rhabdome, which is very clearly seen in cross sections through the ommatidia (Fig. 11). The cross sections show in addition that another cell division has occurred, since they now show eight instead of four cells in each ommatidial precursor. In the next 24 hr. the ommatidia differentiate quite rapidly assuming very nearly their definitive form (Fig. 13), undergoing only further elongation in subsequent development. At this time, the description of the adult eye published by Johannsen (8) or Hertweck (6) is applicable without change except as to length and pigmentation.

Bar

Re-examination of the eye disks of Bar larvae showed that the "goblets" (the structures seen when the ommatidial precursors are cut longitudinally) were present in them just as in wild type. They made their first appearance at 72 hr. after hatching (Fig. 15) and as in the wild type increased in number between 72 and 96 hr. of larval life (Fig. 17). The number of "goblets" in the Bar eye disks is many fewer than that in the wild type. For example, at no time were more than four "goblets" seen in any one section of Bar eye disks while more than 20 were seen in a single section of a wild type eye disk. The low frequency with which the "goblets" (ommatidial precursors) are present in Bar accounts for the author's failure to detect the "clusters" (the structures seen when the ommatidial precursors are cut in cross section) in the Bar eye disks, while they were readily seen in the wild type (19). A renewed search for the "clusters" again resulted in failure, or at least uncertainty. This may be expected since a section through four ommatidial precursors would result in a group of four cell clusters, a figure that may easily be confused with a shrinkage artifact. It is precisely because of this difficulty that the author hesitates to say with certainy that the "clusters" have been seen in Bar eye disks, nevertheless, it is obvious that they must have been present in some preparations since it is unlikely that there were no cross sections through the ommatidial precursors. In their subsequent development the Bar "goblets" parallel the wild type in every detail (Figs. 18 and 19).

Special attention was paid to the pigmented, non-facetted region of the eye to check its mode of development. This region is of particular importance in the light of Wolsky and Huxley's (25) report that ". . . . . the (pigmented) non-facetted regions seem to be rather degenerate in their structure. . . . . the non-facetted area consists of distinct blocks of rather hypertrophied cells, while between the blocks some narrow space can be distinguished . . . . . these solid blocks of heavily pigmented cells correspond to rudimentary "ommatidia", in which, however, the elements of the ommatidia proper . . . . . are entirely lacking. Thus the blocks consist only of accessory

14

15

16

17

18

19

All prints are contact prints. All magnifications given are initial magnifications at the ocular. B = brain; E = eye disk; L = limb bud.

FIG. 14. *Section through a Bar larva 72 hr. after hatching. 140×.*

FIG. 15. *High power photograph of a portion of the optic disk shown in Fig. 14. 840×. The arrow indicates a "goblet".*

FIG. 16. *Section through a Bar larva 96 hr. after hatching. 140×.*

FIG. 17. *High power photograph of a portion of the right optic disk shown in Fig. 16. 840×. The arrow indicates a "goblet".*

FIG. 18. *Cross section through the eye of a 24 hr. Bar pupa. 840×. The arrow indicates a "goblet" and its nerve.*

FIG. 19. *Cross section through the eye of a 48 hr. old Bar pupa. The future pigmented, unfacetted region is at the right. 840×.*

elements which normally form the pigmented coat around the retinula, but here take its entire place. . . . . in some cases there is no sharp demarcation between the structure of the facetted and non-facetted regions and one can find degenerated ommatidia which still contain some rudiments of the retinula and dioptric apparatus showing also rudimentary facet formation in the overlying cuticle." These observations lend support to those hypotheses requiring a secondary destruction of already formed material.

The pigmented, non-facetted region can easily be recognized at 48 hr. after puparium formation. At this time it appears as a compactly organized tissue with no evidence of organization into ommatidial precursors (Fig. 19). Many slides were examined and this point was checked and rechecked. At 72 hr. after puparium formation the only changes that the pigmented, non-facetted region has undergone are (*a*) a very heavy deposition of pigment and (*b*) an elongation. As at 48 hr., there is no evidence of degenerate ommatidia; *there are no ommatidia in this region at any time.* It seems quite likely that the "degenerate ommatidia" that Wolsky and Huxley believed they saw were simply oblique sections through a portion of some ommatidia that border the non-facetted region. The author has seen such sections in his own preparations. However, if several successive sections are studied, the nature of the structures becomes clear.

Although slides of 30-, 36-, and 42-hr. pupae were studied with the hope of obtaining an insight into the origin of the pigmented, non-facetted region, no satisfactory evidence as to the origin of this region was obtained. Undoubtedly further study would be rewarded with success but since this question is not pertinent to the problem under investigation it was not pursued further.

Some Observations on the Eversion of the Cephalic Complex

During these investigations some observations on the course of the evagination of the cephalic complex were made that may serve to supplement those reported by Robertson (18). During the prepupal period the position of the optic disk changes from its original one of close apposition to the brain to a position just within the anterior opening of the frontal sac (Fig. 7). This new position is attained by the gradual rotation of the disk anteriorly. It reaches its most advanced point at about nine hours after puparium formation. Sometime between 11½ and 12 hr. after puparium formation, the disks are suddenly everted (Robertson, 18 and the author). During these manoeuvers of the optic disk, the optic nerve follows faithfully along. This is clearly shown in Fig. 7. It seems clear therefore that the nervous connection established at least as early as 24 hr. after hatching from the egg (19) is maintained throughout the remainder of development. The situation described here is identical with that described by Van Rees (22) in *Musca vomitoria* and by Miall and Hammond (13) in *Chironomus*. As a matter of fact, the figures and descriptions of the metamorphosis of the head given in these papers may be applied unchanged to *Drosophila*.

What remains unclear is the origin of the nerve fibres connecting the ommatidia and the brain. The optic stalk is established long before the ommatidial precursors can be seen. It increases in thickness as development proceeds, i.e. more nerve fibres develop. Moreover there is a clear relationship between the number of ommatidia and the number of fibres that develop. This is most sharply brought out by the demonstration that the fewer the ommatidia the greater the reduction in the optic ganglia of the imago (8 and 17). The fact that there is no associated reduction in the larval dorsal ganglia (12 and 14) is not surprising since there are no true optic ganglia in the larval brain (6).

The nerve fibres may originate in the larval brain and then make connection with the ommatidia. Alternately the fibres may originate at the ommatidia and then grow toward the brain. There are no data available at present that would aid in deciding between these alternatives. The fact that in *Drosophila* the optic disk will develop normally when transplanted without any connection to the brain, may afford a method of deciding the question. It would be necessary to transplant disks from very young larvae (e.g. 24 hr. after hatching) and then study the innervation of the implanted eye after metamorphosis. Pilkington (14) by transplanting eye disks of mature larvae into similar hosts, obtained confirmation of Weismann's suggestion that the outer optic ganglion forms from the optic stalk. Transplantation at this stage, however, is much too late to study the origin of the nerves since they are already present and are to some extent organized into a ganglion.

Discussion

The data presented above show that the histological differentiation of the "clusters" and "goblets", depending on the plane of section, begins between 48 and 72 hr. after hatching at 25° C. in Bar just as in wild type; that thereafter the histological picture is the same in both forms with the exception of the pigmented but unfacetted region of the Bar eye. They show quite definitely that these structures (the "clusters" and "goblets") are the precursors of the ommatidia. Furthermore, the data establish that the pigmented, unfacetted region of the Bar eye does not result from a degeneration of already formed ommatidia.

These facts are further contradictions to those hypotheses that involve a destruction of already formed materials to explain the development of the Bar eye. The histological evidence taken in conjunction with the evidence derived from a study of the growth curves of the eye disks of Bar, modified Bar, and wild type (19) makes it clear that all such hypotheses as well as those involving different growth rates of Bar and wild type must be discarded. On the other hand, these data lend further support to the hypothesis advanced by the author (19 and 20) that the primary morphological effect of the Bar mutation is to reduce the capital of cells going into the formation of the cephalic complex; more specifically, into that portion of the cephalic complex that will eventually give rise to the eye disk. In its complete form the

hypothesis postulates, in addition, the presence of a group of cells labilely determined to form either facets or head chitin. The ultimate fate of these cells depends upon the nature of various extrinsic and intrinsic modifiers that may be present. It is the presence of these labilely determined cells that permits the variation of eye size observed within a given genotype. This hypothesis may suffice to explain the development of other mutants that reduce the facet number of the eye, e.g. eyeless, glass, lobed, etc., however, direct tests are desirable and these tests are under way.

One phase of the problem of the development of the Bar eye remains unexplored, namely, the question of the shape of the eye and the distribution of the facets within it. The present studies shed no light on the origin of the pattern of the Bar eye. It must be emphasized, however, that a complete explanation of the mode of development of the Bar eye will not be available until this phase of the problem is understood. This is equally true of all other mutations that affect facet number.

The author recognizes that his hypothesis falls short in that it does not explain the origin of the Bar pattern. Nevertheless, it is necessary to explain the method of facet number reduction as a preliminary to an explanation of the pattern woven out of this reduced number of facets. Studies such as the one reported above are directed toward that end.

References

1. AUERBACH, C. Trans. Roy. Soc. Edinburgh, 58 : 787-815. 1936.
2. BODENSTEIN, D. Arch. Entwicklungsmech. Organ. 137 : 474-505. 1938.
3. CHEN, T. Y. J. Morphol. Physiol. 47 : 135-199. 1929.
4. ENZMANN, E. V. and HASKINS, C. P. J. Morphol. 63 : 63-73. 1938.
5. GOLDSCHMIDT, R. Univ. Calif. Pub. Zool. 41 : 277-282. 1937.
6. HERTWECK, H. Z. wiss. Zool. 139 : 559-663. 1931.
7. HUXLEY, J. S. Biol. Zhur. 4 : 421-425. 1935.
8. JOHANNSEN, O. A. J. Morphol. Physiol. 39 : 337-349. 1924.
9. KALISS, N. Genetics, 24 : 244-270. 1939.
10. KRAFKA, J., JR. Biol. Bull. 47 : 143-148. 1924.
11. MARGOLIS, O. S. and ROBERTSON, C. W. Genetics, 22 : 319-331. 1937.
12. MEDVEDEV, N. N. Z. ind. Abst. Vererb. 70 : 55-72. 1935.
13. MIALL, L. C. and HAMMOND, A. R. Trans. Linnean Soc. Zool. (Ser. 2), 5 : 265-280. 1892.
14. PILKINGTON, R. W. Proc. Zool. Soc. London (Ser. A), 111 : 199-222. 1941.
15. POULSON, D. F. Actualités sci. 498 : 1-51. 1937.
16. POULSON, D. F. J. Exptl. Zool. 83 : 271-325. 1940.
17. RICHARDS, M. H. and FURROW, E. Y. Biol. Bull. 48 : 243-258. 1925.
18. ROBERTSON, C. W. J. Morphol. 59 : 351-399. 1936.
19. STEINBERG, A. G. Genetics, 26 : 325-346. 1941.
20. STEINBERG, A. G. Genetics, 26 : 440-451. 1941.
21. STEINBERG, A. G. Genetics, 27 : 171-172. 1942.
22. VAN REES, J. Zool. Jahrb. 3 : 1-134. 1889.
23. WADDINGTON, C. H. J. Genetics, 41 : 75-139. 1940.
24. WEISMANN, A. Z. wiss. Zool. 14 : 187-336. 1864.
25. WOLSKY, A. and HUXLEY, J. S. Proc. Zool. Soc. London, Pt. II : 485-489. 1936.

STUDIES ON CESTODES OF THE GENUS *TRIAENOPHORUS* FROM FISH OF LESSER SLAVE LAKE, ALBERTA

II. THE EGGS, CORACIDIA, AND LIFE IN THE FIRST INTERMEDIATE HOST OF *TRIAENOPHORUS CRASSUS* FOREL AND *T. NODULOSUS* (PALLAS)[1]

By Richard B. Miller[2]

Abstract

The eggs of *Triaenophorus crassus* vary from 53 to 68 μ long by 38 to 44 μ in diameter. Those of *T. nodulosus* are practically the same ranging from 58 to 67 μ long by 38 to 44 μ in diameter. Movement of the embryo within the egg is discernible two days before hatching. The eggs of one individual hatch over a period of eight to 10 days.

The coracidia of *T. crassus* are from 67 to 80 μ long by 49 to 58 μ wide and of *T. nodulosus* from 67 to 85 μ long by 58 to 80 μ in diameter. The coracidia live for two or three days and are free swimming for most of this time.

The first intermediate host of both species in Lesser Slave Lake is *Cyclops bicuspidatus* Claus; the coracidia of both species will also infect *Diaptomus ashlandi* Marsh, but fail to develop in this copepod. The procercoids develop in the body cavity of the *Cyclops* in eight to 10 days after their entry; both species attain in this time an average length of 300 μ. Where a large number of procercoids occurs simultaneously in one *Cyclops* the growth rate is slower and the maximum size attained is less.

The Eggs and Hatching

In Part I of these studies (4) the maturation of *T. crassus* and *T. nodulosus* was described, and the times and manner of egg-laying of each detailed. For the former the egg-laying period is the first half of May; for the latter it is mostly in early June, about one month later.

The eggs of both species are white in colour but turn brown about 45 min. after being placed in water. They are "egg-shaped" and nearly the same size; in *T. crassus* they vary from 53 to 68 μ long by 38 to 44 μ in diameter (average 61 $\times$ 41 μ) and in *T. nodulosus* from 58 to 67 μ long by 38 to 44 μ in diameter (average 61 $\times$ 44 μ).

In the ripe eggs the onchospheres can be clearly made out (Fig. 1). Two days before eclosion faint movements of the embryo are discernible. These become more and more violent and culminate in the popping open of the operculum at the small end of the egg and the emergence of the coracidium. The round aperture revealed when the operculum opens is much smaller in diameter than the coracidium which is forced to struggle considerably to pass through it; hatching usually lasts from 15 to 30 sec. Several times coracidia were seen that had failed to get entirely out of the egg and were swimming around dragging it with them. Sometimes the operculum bursts completely off the egg during hatching but in most eggs it remains attached by one side (Fig. 2).

[1] *Manuscript received June 11, 1943.*
Contribution from the Department of Zoology, University of Alberta, Edmonton, Alta.
[2] *Lecturer in Zoology.*

FIG. 1. *Photomicrographs of two living eggs of Triaenophorus crassus just prior to hatching; the outlines of the coracidia can be seen through the transparent egg shells. Upper egg magnified 148 times, lower 189 times.*

FIG. 2. *Photomicrograph of the empty egg of Triaenophorus crassus; the operculum and the hole through which the coracidium escaped can be seen. Shown also is an unripe, probably dead, egg.* ×*148.*

FIG. 3. *Photomicrograph of the living coracidium of T. crassus about two hours old.* ×*189.*

FIG. 4. *Photomicrograph of a stained whole mount of Cyclops bicuspidatus containing two mature procercoids of Triaenophorus crassus.* ×*60.*

FIG. 5. *The anterior procercoid of the two shown in Fig. 4 photographed through an oil immersion lens; the cercomere and two of its hooks and the invagination in the future scolex region can be distinguished.* ×*390.*

Detailed observations were made on the hatching of the eggs from over a dozen different specimens of *T. crassus*. The eggs from each worm were placed in a Petri dish and covered with approximately 1 cm. of water. Every day at about the same hour the eggs were stirred and a sample pipetted to a glass slide and examined microscopically. With the aid of a Whipple disk, counts of the relative numbers of hatched and dead eggs were made. At the same time the numbers of living and dead coracidia were estimated. The results of some of these observations are shown in Table I.

TABLE I

THE DURATION OF HATCHING AND LENGTH OF LIFE OF THE CORACIDIA AS OBSERVED IN FIVE CULTURES OF EGGS OF *T. crassus*

Days after incidence of hatching	0	1	2	3	4	5	6	7	8	9	10
Culture 1											
Eggs hatched, %	0.1	4	30	60	52	81	84	86	—	—	94
Eggs dead, %	—	—	—	—	—	—	17	6	—	—	6
Coracidia dead, %	—	—	—	—	—	—	—	—	—	100	100
Culture 2											
Eggs hatched, %	1.7	12	16	14	48	41	59	48	56	50	—
Eggs dead, %	—	—	—	—	—	24	29	40	43	50	—
Coracidia dead, %	—	0	16.7	25	20	12	33	76	88	100	—
Culture 3											
Eggs hatched, %	9	12	37	54	61	70	—	—	75	—	—
Eggs dead, %	—	—	—	20	30	28	—	—	25	—	—
Coracidia dead, %	—	0.2	41	27	41	70	60	—	75	—	100
Culture 4											
Eggs hatched, %	0.1	—	78	—	74	—	86	—	98	—	—
Eggs dead, %	—	—	—	—	—	—	—	—	—	—	—
Coracidia dead, %	—	—	38	—	19	—	56	—	100	—	100
Culture 5											
Eggs hatched, %	0.1	2.9	5.8	11.5	42.7	50	50	74	55	61	74
Eggs dead, %	—	—	—	—	—	26	35	25	43	38	25
Coracidia dead, %	—	—	—	—	—	—	69	85	97	96	100

The temperature in the room where the cultures of eggs were kept varied from 55° to 70° F.; most of the time it remained in the fifties.

Hatching continued for as long as 10 days after it began; in some cultures half the eggs had hatched by the third day and usually most of the viable eggs had hatched by the end of a week. Cultures of the eggs of *T. nodulosus* were much the same; hatching usually continued for eight days and occasionally for 10.

Noteworthy is the high viability of some of the cultures of *T. crassus* eggs; a fully ripe adult of *T. crassus* was estimated to contain 1,175,000 eggs; in one culture 94% of all these eggs hatched; in another, believed to be exceptional, only 50% of the eggs proved to be viable. Many cultures for which

no accurate records were kept appeared to have very few dead eggs when hatching was completed.

The data, here reported, on the dimensions of the eggs of *T. crassus* and the duration of hatching are in agreement with similar data published by Ekbaum (1) for her Canadian material.

The Coracidia

Immediately after eclosion the coracidia are about the same size as the egg, sometimes smaller. For about an hour they swim so rapidly that it is impossible to catch more than fleeting glimpses of them in the field of a compound microscope. During this period they gradually increase in size. This increase is due to the swelling of the single layer of ciliated cells with which each is invested; the actual onchosphere remains the same size as it was in the egg just prior to hatching. When their full proportions are attained their movements are quite slow and they can be readily observed. They are shaped rather like a hen's egg and swim with the broader, hook-free end anterior. During forward movement they rotate slowly first in one direction, then in the other, alternating irregularly. The cilia responsible for this movement can be made out with the high powers of a microscope, especially with the aid of a dark field condenser. The present study was made in the field and proper facilities for such work were not available; Dr. Ekbaum's measurements of the cilia of the coracidia of *T. crassus* are quoted here. She states these to be 10 to 15 μ long with a special tuft 30 to 35 μ long at the end directed forward when the coracidia are swimming (1). Observations the author was able to make are in agreement with these findings.

Coracidia of *T. crassus*, one day old, ranged in size from 67 to 80 μ long by 49 to 58 μ wide (average 73 $\times$ 54 μ) (Figs. 3 and 6). Ekbaum (1) found

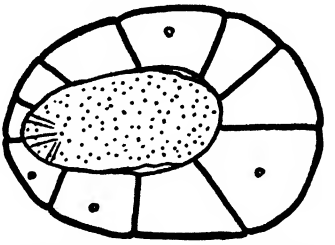


FIG. 6. *Camera lucida drawing of a two day old coracidium of Triaenophorus crassus. The cilia are not shown.* $\times 600$.

the coracidia of *T. crassus* to measure 60 to 70 μ in length and 50 to 60 μ in diameter. Newton (5) stated that the coracidia of *T. tricuspidatus* (presumably *T. crassus*) "are extremely small, measuring six μ in diameter". Furthermore he states that the coracidia appeared 15 days after placing the eggs in water; in the experience of the present investigator the eggs would be dead after this lapse of time. Newton's coracidia also failed to infect various

species of copepods that were exposed to them; he states that, ". . . . . the coracidia remained alive for over a month . . . . .". This is a much longer coracidial life span than has been found by other workers and in the present study. In view of these discrepancies it is possible that Newton measured organisms of some kind that developed in his culture and that were not coracidia.

Coracidia of *T. nodulosus* are of nearly the same proportions as those of *T. crassus*; they tend to be somewhat broader, ranging from 67 to 85 μ long by 58 to 80 μ wide (average 75 $\times$ 68 μ).

In Table I the percentage of dead coracidia found each day in some of the egg cultures of *T. crassus* is shown. A few coracidia appear to live for only 24 hr. Most of them probably live for at least twice this time. During most of its life the coracidium is free swimming; before dying it settles on the bottom. A few coracidia were observed to use their hooks to work their way out of their ciliated coats; they then crept around slowly on the bottom of the culture dish for a short time. The majority, after settling, died within their ciliated investment. These observations apply particularly to *T. crassus* but *T. nodulosus* is the same in every respect.

Ekbaum (1) states that "after three to four days the coracidium gradually loses its cilia, ceases the movements and remains on the bottom of the dish. In several cases it was noted that the outer covering with the cilia was cast off." These observations on *T. crassus* are essentially the same as those reported here.

The power of infecting the first intermediate host was tested at different times during the period that a culture still possessed live, unhatched eggs and living coracidia. During the week following the incidence of hatching the egg and coracidia cultures of *T. crassus* were capable of infecting close to 100% of the first intermediate hosts that were exposed to them. The latest successful infections were obtained with cultures eight days after hatching began; in this experiment only two out of 22 *Cyclops* became infected; living onchospheres were found in pellets defecated by some of the *Cyclops* in these cultures; they had evidently been swallowed by the *Cyclops* but lacked the strength to burrow through the gut wall into the body cavity.

Development of the Procercoid in the First Intermediate Host

The field laboratory set up for this study was on the shore of Lesser Slave Lake; plankton was secured from the lake as the need arose and inoculated with egg and coracidia cultures of *T. crassus* and *T. nodulosus*. The two most abundant plankters in the shallow waters were the copepods *Cyclops bicuspidatus* Claus and *Diaptomus ashlandi* Marsh. Both of these became infected with either *T. crassus* or *T. nodulosus*, but *Cyclops bicuspidatus* is the natural host; in none of the experiments was a procercoid of either species observed to reach maturity or grow to any appreciable extent in *Diaptomus*. Two other copepods, *Cyclops albidus* Jurine and *C. viridis* Jurine were also

tried as possible hosts. No infections of *C. albidus* with either *T. crassus* or *T. nodulosus* were observed and only one *C. viridis* was found that contained a parasite (young procercoid of *T. nodulosus*). Various cladocerans including *Daphnia pulex* (de Geer) and *D. longispina* (O. F. Müller) proved immune to infection by either species of parasite.

Michajlow (3) made a detailed study of the ability of coracidia of *T. nodulosus* to infect different species of copepods. (This paper is known to the author only through an abstract.) He found five types of copepod as follows:—

1. The digestive juice of the copepod killed the coracidia in the gut : *Diaptomus amblyodon* Maren, *D. castor* Jurine, and *Cyclops viridis* Jurine.

2. Most of the parasites killed by digestive juice but a few get into the body cavity: *Cyclops orthonoides* Sar and *C. leuckarti* Claus.

3. Majority of the coracidia enter the body cavity but fail to develop: *Cyclops serrulatus* Fischer.

4. A few parasites get into the body cavity and develop normally: *Cyclops albidus* Jurine, *C. insignis* Claus, *C. fuscus* Jurine, *C. vernalis* Fischer, *C. bicuspidatus* Claus, and *Diaptomus gracilis* Sar.

5. All coracidia get from gut to body cavity and develop normally: *Cyclops strenuus* Fischer.

Cyclops strenuus, the natural host in Europe, was not found by the author in any of the Alberta lakes investigated (14 lakes). *Cyclops bicuspidatus*, however, was abundant in all of them. Usually all the coracidia swallowed by this copepod successfully negotiated the gut wall but occasionally some were seen to be defecated. The coracidia of both *T. crassus* and *T. nodulosus* were most successful in establishing themselves in the nauplii and young adults. *Diaptomus ashlandi* would seem to belong in Michajlow's third group according to the observations recorded here.

The study of the development of the procercoids of both *T. crassus* and *T. nodulosus* was made using *Cyclops bicuspidatus* as the host. Within one hour of the time of adding coracidia of *T. crassus* to a culture of these copepods the parasites could be found in their stomachs and body cavities; each *Cyclops* usually contained four or five by this time; of these one or two were in the body cavity and the remainder were still in the stomach where they could be observed digging through the stomach wall. All the parasites had lost their ciliated coats. The interesting process of penetrating the stomach wall was observed in some detail; each parasite swings its six hooks backward in such a manner that the ends come together to form one pointed tool; this point is then thrust into the stomach wall a short distance; the hooks are then slowly and forcefully spread apart so as to enlarge the breach in the wall. This process is repeated several times until a large enough hole is torn in the stomach for the parasite to wriggle through into the perihaemal space of the *Cyclops*. At each thrust of the hooks the stomach muscles of the *Cyclops* contract violently; often the animal's whole body gives obvious signs of

distress by spasmodic jerkings, particularly of the abdomen and antennae. On one occasion the contractions of the *Cyclops*' stomach were so violent that all the parasites contained in it were forcibly ejected through the mouth and back into the water, where they crawled about for a short time and then died.

The use of the coracidial hooks described in the preceding paragraph appears to be the only one; the hooks were often moved afterward, but were never put to any apparent use.

The subsequent growth of the parasite from the coracidium to the mature procercoid was observed in many hundreds of *Cyclops* for both *T. crassus* and *T. nodulosus*. More careful records of these observations were kept for *T. crassus* and a summary of them for three cultures, two lightly and one heavily infected, is shown in Table II. While these observations apply particularly to *T. crassus*, the facts are practically the same for the other species.

TABLE II

THE GROWTH OF *Triaenophorus crassus* FROM THE CORACIDIUM TO THE MATURE PROCERCOID IN THE BODY CAVITY OF *Cyclops bicuspidatus*[1]

	Age of procercoid, days								
	1	2	3	4	5	6	7	8	9
	Length, μ								
Culture 1[3]	—	62	—	—	—	155	—	310[2]	—
Culture 2[4]	50	80	—	110	—	220	—	352[2]	—
Culture 3[5]	—	80	75	80	—	160	175	89	220
	Age of procercoid, days								
	10	11	12	13	14	15	16	17	
	Length, μ								
Culture 1	—	220[2]	—	—	—	—	—	185[2]	
Culture 2	330[2]	—	310[2]	—	—	265[2]	—	—	
Culture 3	200[2]	—	—	—	200[2]	—	—	—	

[1] *The figures in the table are the lengths in μ of the largest procercoids.*
[2] *Indicates that a cercomere was developed.*
[3] *Culture 1, average of 2.7 procercoids per Cyclops.*
[4] *Culture 2, average of 2.8 procercoids per Cyclops.*
[5] *Culture 3, average of 10 procercoids per Cyclops.*

When the coracidium first arrives in the body cavity of the *Cyclops* it is the same size as it was as a free living organism within its ciliated investment. After two days in the *Cyclops* a small increase in size can be noticed. Subsequent growth is rapid; by four days its size is doubled and by eight days it has reached its mature size of 300 to 350 μ, roughly six times the length of the coracidium. The procercoid was taken as mature when the cercomere, or caudal appendage, was clearly pinched off and the terminal invagination (frontal gland) in the future scolex region could be distinguished (Figs. 4

and 5). Also visible was the complex excretory system leading to an invagination at the posterior end where the stalk of the cercomere is attached; eight symmetrically disposed calcareous particles, four in an anteroposterior line on each side, were usually discernible as well. The length of the procercoid at this stage varied from 55 to 352 μ, but was usually close to 300 μ. The cercomere varied from 25 to 50 μ in length.

After the cercomere is established the procercoid either stops growing or grows very slowly. Cultures of copepods were kept alive for as long as 22 days after infection with coracidia of *T. crassus*; no increase of size of the procercoids was observed after the eighth day.

The number of parasites per host has some effect on the growth rate. This number ranged from 1 to 32 in a single *Cyclops*. Two of the cultures recorded in Table II averaged 2.7 and 2.8 procercoids per *Cyclops*; procercoids in these reached their maximum size in eight days. In the third culture in Table II the average number of developing procercoids per *Cyclops* was 10. In this culture the growth of the procercoids was little affected until after the fourth day; the overcrowding factor then began to operate; mature procercoids were not found until the 10th day, two days later than in the less heavily infected cultures; the largest found measured only 200 μ, about two-thirds the size attained in the other cultures.

During the whole of their lives within the *Cyclops* the procercoids display active movement; they are never attached to any part of the host's body. They constantly lengthen and shorten, gliding from one part of the host to another. Young procercoids were frequently found in the antennae and caudal rami as well as the more usual locations in the thorax and abdomen. Their presence seemed not to affect the *Cyclops* appreciably. As mentioned above parasitized *Cyclops* lived for a period of 22 days and might have lived longer had they been free from the rough handling involved in microscopic examination. Janicki (2) noted that *Cyclops* heavily infected with procercoids of *T. nodulosus* tended to settle to the bottom of their culture dish and become covered with epiphytes. This was noted in the present investigation for *Cyclops* heavily parasitized with either *T. crassus* or *T. nodulosus*.

Procercoids of either species have never been observed to lose their cercomeres while still in the body of the host. This appendage is very lightly attached, however, as a slight pressure on the coverslip during microscopic examination will cause it to drop off.

The number of procercoids of *T. nodulosus* per *Cyclops* was observed to vary from 1 to 27. The development of the procercoid is essentially the same as described above for *T. crassus*. Janicki (2) found in his studies of *T. nodulosus* in *Cyclops strenuus* that the procercoid stage was reached in 11 days. The maximum size of the procercoids in his investigation was 714 μ attained at the end of a month. In the present study procercoids of *T. nodulosus* reached only a little over half this size in about 20 days. The fact, mentioned above, that Michajlow has shown that *Cyclops bicuspidatus* is not the natural host for this species may explain this difference in the size of the procercoid.

Acknowledgments

In addition to the help received from the Fisheries Branch of the Government of the Province of Alberta, acknowledged in Part I of these studies, the author would like to express his gratitude to Mr. J. E. Moore, formerly of the Department of Biology, University of Toronto, now of the R.C.A.F. Mr. Moore kindly checked the author's identification of the copepods from Lesser Slave Lake.

References

1. EKBAUM, E. J. Parasitol. 23 : 293-295. 1937.
2. JANICKI, C. Korresp.-Bl. Schweiz. Aerzte, 48 : 1343-1349. 1918.
3. MICHAJLOW, W. Ann. parasitol. humaine comp. 10 : 334-344. 1932.
4. MILLER, R. B. Can. J. Research, D, 21 : 160-170. 1943.
5. NEWTON, M. V. B. Contrib. Can. Biol. Fisheries (n.s.), 7 (28) : 341-360. 1932.

VARIATION IN THE PORCUPINE (GENUS *ERETHIZON*) IN CANADA[1]

By R. M. Anderson[2] and A. L. Rand[3]

Abstract

The porcupines of Canada (genus *Erethizon*) have usually been referred to two distinct species. A survey of material in the National Museum of Canada shows that they actually represent but one species, *Erethizon dorsatum*, with five geographical races, which are summarized in this paper. Each form is differentiated by a mosaic of external and internal characters, some of which frequently occur in other forms. There is considerable individual variation. Some overlapping of characters through individual variation occurs in widely separated populations. Where two rather distinct subspecies meet, intergrades occur, but intergradations in skull characters do not always occur in the same geographical area as intergradations of pelage characters.

Introduction

The porcupines of Canada present some interesting aspects of variation in both pelage and skull characters. Hollister (9, p. 27), considering specimens from Jasper National Park, says that the skulls of this porcupine exhibit more individual variation than do those of any other mammal that he has carefully studied. However, individual variation appears to be greater in some areas than in others, as he says Alaska specimens are remarkably uniform throughout a large series. Swarth (15, pp. 380–381) discussing Skeena River specimens mentions variability in colour, but suggests that cranial characters vary geographically. Bangs (3, pp. 37–39) mentions the variability in both skin and skulls of eastern porcupines, but considered it in part correlated with geography. Allen (1, p. 391) has pointed out the variation in skulls from Maine.

Our material shows that while in some populations there is great variation, as in the pelage of Teslin Lake specimens and in the skulls from southern British Columbia, in others there is much less variation, as in the pelage of skins from Eastern Canada, and in skulls from the Bella Coola area of British Columbia, and that some variation is geographical.

Two species are currently recognized in the genus *Erethizon*, both occurring in Canada, a black-haired eastern porcupine, *E. dorsatum* Linnaeus (10, p. 57), with two subspecies, and a yellow-haired western porcupine, *E. epixanthum* Brandt (5, p. 390), with several subspecies.

The yellow-haired porcupine has been considered as ranging over the plains and mountains from Turtle Mountains, N.D. and Wood Buffalo Park westward, and the black-haired porcupine, mainly to the east of that but occasionally occurring as far west as northern British Columbia, its range thus overlapping that of the yellow-haired form.

[1] *Manuscript received June 11, 1943.*
Contribution from the Division of Biology, National Museum of Canada, Ottawa, Canada.
[2] *Chief, Division of Biology, National Museum of Canada.*
[3] *Assistant Zoologist, National Museum of Canada.*

Porcupines are awkward things to handle, and this has retarded building up of series of specimens and their study. In the National Museum of Canada we have a series of some 63 skins and 71 skulls from many parts of Canada. There still are areas from which we need specimens to determine the limits of forms and the status of populations. Especially do we need material from the southern part of the Prairie Provinces, from the area between northern Manitoba and the Yukon, and from Ungava. But a survey of the material available brings out some interesting points.

It appears that the yellow-haired and the black-haired porcupines intergrade; in pelage notably in the southern Yukon, in the vicinity of Teslin Lake, and in skull characters in the Wood Buffalo Park area at least. This makes it necessary to consider the porcupines of the genus *Erethizon* as belonging to one species instead of two, and the names of the forms (see Fig. 1) occurring in Canada should stand as follows:

A. Erethizon dorsatum picinum Bangs (1900)
B. Erethizon dorsatum dorsatum (Linnaeus) (1758)
C. Erethizon dorsatum myops Merriam (1900)
D. Erethizon dorsatum nigrescens Allen (1903)
E. Erethizon dorsatum epixanthum Brandt (1835)

Two additional forms have been described as subspecies of *Erethizon epixanthum* and are admitted as such to the North American list by Miller (13, p. 437), namely:

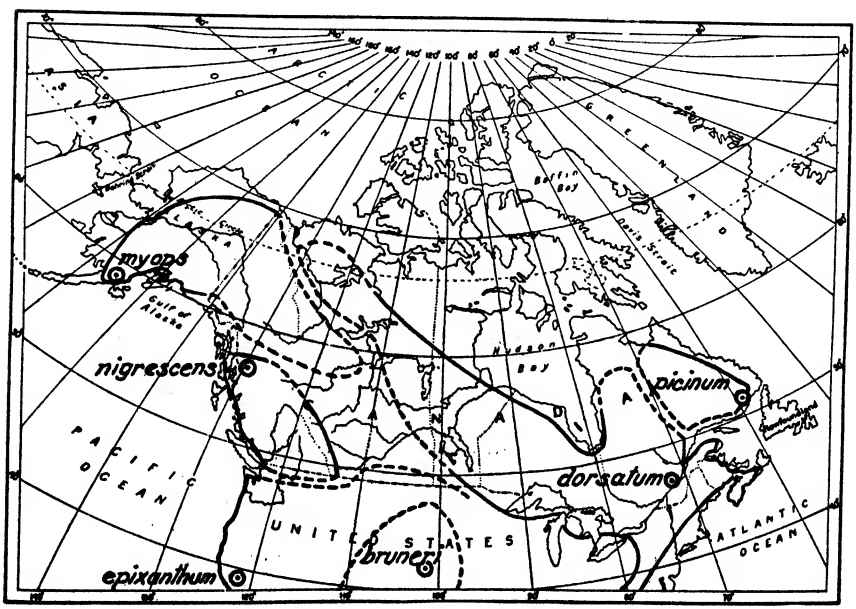


FIG. 1. *Geographic distribution of the races of Canada porcupine (Erethizon dorsatum) in Canada.*

1916. *Erethizon epixanthum bruneri* Swenk (17, pp. 115–125). Type locality, three miles east of Mitchell, Scottsbluff County, Neb. (near the Wyoming boundary). Nebraska porcupine.

1897. *Erethizon epixanthum couesi* Mearns (11, p. 723). Type locality, Fort Whipple, Yavapai County, Ariz. Arizona porcupine.

Both *bruneri* and *couesi* are described as paler and duller than the typical *E. epixanthum* of California, *bruneri* being slightly larger and *couesi* slightly smaller than *epixanthum*. The subspecies *bruneri*, if considered a tenable form, may be regarded as of doubtful occurrence in Canada, and *couesi*, the most southern race of the genus, is extralimital to Canada.

Allen (1, pp. 388–389) considered the two forms that he recognized, *dorsatum* and *epixanthum*, as varieties of one species and showed that they intergraded. Later, when he described *nigrescens* (2, p. 558) he considered it a race of the separate species *epixanthum*.

From a survey of our material the more important features that vary geographically were found to be as follows.

1. The general tone of the dark pigmented fur, from brownish to blackish.
2. The abundance of long guard hairs.
3. The abundance of yellowish or whitish tips to the guard hairs, and the width of these light tips.
4. The extent of whitish coloration on the head.
5. The shade of the whitish or yellowish coloration.
6. The extent and type of ridging on the cranium in the adult skulls.
7. The amount of swelling in the frontal bones.
8. The amount of depression in the frontal-parietal area.
9. The length of the nasals, and the shape of their posterior margin.
10. The development of a ridge on the sides of the premaxilla.

While all these characters vary geographically, they do not form an even trend in variation from one end of the population to the other; that is they do not represent a cline; intergradation appears to occur on a small part of the range only. There is also pronounced individual variation, so that individuals from widely separated populations often approach each other in some characters. Before going on to diagnose the recognizable forms in detail it may be advisable to discuss the variation in our series as a whole.

Explanation of Measurements

External Measurements

External measurements are in millimetres and are those made by the collector from the animal in the flesh. The following have been used:

Total Length.—The distance in a *straight line* from the tip of the nose to the end of the last tail vertebrae, exclusive of the hairs; animal lying on its back with the body stretched and the limbs pulled into a natural position.

Tail Vertebrae.—The distance from base of tail to tip of the terminal vertebra, exclusive of the hairs.

Hind Foot.—The distance from the end of heel bone (calcaneum) to the end of claw on the longest toe. The toes should be straightened out and this may be done by pressing them flat against the ruler, or the foot may be pressed flat on the table and the length measured with dividers.

Skull Measurements

Skull measurements are in millimetres and are made with caliper or dividers from clean, dry skull. The following have been selected as the most useful for making comparisons of specimens in this particular species:

Condylobasal Length.—Distance from the posterior border of the occipital condyle to the posterior edge of the alveolus of the incisor on the corresponding side of the skull.

Zygomatic Breadth.—Distance between the zygomatic arches at their widest point.

Length of Nasals.—From the most anterior point to most posterior point.

Width of Rostrum.—Greatest breadth of rostrum measured across anterior borders of the anteorbital foramina.

Age of course changes the appearance of skulls tremendously, so that only adult skulls are compared. Female skulls usually have the characters less pronounced, and must be compared separately. However we have few female skulls suitable for study. There is little difference in pelage between the sexes; in size females average smaller, but for external measurements it must be considered that porcupines are very difficult to handle and measure, and a high variability in measurements is to be expected; seasonal variation chiefly affects the under fur; it is long and completely conceals the spines in winter, short and scanty, leaving the spines mostly exposed in summer.

Albinos are not included in the present discussion. They are of occasional occurrence probably throughout the range of the species. We have specimens as follows:

Yukon: Whitehorse; partial albino, spines and fur white, except for broad rusty tips to guard hairs, nails whitish.

Alberta: Knee Hill Valley; partial albino, spines and fur white except for pale rusty tips to guard hairs, nails dusky.

Ontario: Ottawa; partial albino, white with a slight grey tinge in the pelage of dorsum, claws whitish.

Quebec: Near Chimo, south of Ungava Bay, a nearly pure white albino skin, without skull, was taken by natives and presented to the National Museum by Hudson's Bay Company through Mr. John Carroll of the Bureau of Geology and Topography in June, 1943.

We also have unpublished manuscript notes of a white porcupine from New Brunswick and two from western Quebec.

Schultz (14) recorded that in 53 porcupine skulls examined by him, 27 had bregmatic fontanelle bones present. In our series 62 skulls have the top of the skull complete, and only 10 of these show such extra bones. It should be pointed out that about half of the skulls in our series have the sutures obliterated, and, when younger, some may have had such accessory bones evident. Of our 10 specimens with bregmatic fontanelle bones, three had one; five had two; one had nine small ones in a cluster, and one had 10 small ones in a cluster.

TABLE I

DISTRIBUTION OF THE THREE COLOUR CHARACTERS OF THE PORCUPINE IN CANADA[1]

Locality	General ground colour[2]				Tint of yellow[3]					Extent of yellow[4]				
	Bl.	Br.Bl.	Bl.Br.	Br.	w.y.	p.y.	r.y.	o.y.	g.y.	A	B	C	D	E
Nova Scotia, New Brunswick, Gaspé (Quebec)	12				10	2				6	5	1		
Western Quebec, Ontario	8				8					4	3	1		
Manitoba		2			1	1					1	1		
Wood Buffalo Park, Alberta		1					1						1	
British Columbia and Jasper Park (Alberta)	14	1			1	4	2	8		1	6	6	3	
Yukon and Alaska	2	4	5	7		6	13			3	3	4	3	8
Cypress Hills (Alberta and Saskatchewan)			2	3	1	1			3		1	1	3	

[1] *The numbers represent the number of individuals, from the locality indicated at the left, with the character indicated at the top of the column.*

[2] *General tone of the under fur and guard hairs where not yellowish; Bl. = blackish, Br. Bl. = brownish black, Bl. Br. = blackish brown, Br. = brownish.*

[3] *Shade of yellowish tips to the guard hairs; w.y. = whitish, p.y. = pale yellow, r.y. = rusty yellow, o.y. = orange yellow, g.y. = greenish yellow.*

[4] *Amount of yellowish tipping; A: yellowish tips confined to nape, hips, and tail; B: yellowish tips also sparingly scattered over back; C: yellowish tips fairly well scattered over back but narrow; D: yellowish tips abundantly scattered over back but narrow; E: yellowish tips abundantly scattered over back and wide.*

A Survey of Variation (See Table I)

Pelage

From Ontario eastward porcupines are brownish black to black with pale yellow to yellowish-white tipping to the guard hairs; this is usually confined to nape, hips, and tail but in occasional specimens is sparingly scattered over the back.

Two northwest Manitoba specimens are more brownish with yellowish-white to yellowish guard hairs more or less sparingly scattered over the back.

A Wood Buffalo Park specimen is blackish, with abundant guard hairs, plentifully tipped with yellow.

A southern Yukon and Chitina River (Alaska) series is more variable; nearly half are brownish with profuse yellow guard hairs, giving a brownish yellow appearing animal; one-quarter are brownish black with few or no

yellowish-tipped guard hairs on the back and are barely distinguishable from eastern animals; one-quarter are intermediate between the above conditions.

British Columbia specimens and one Jasper Park specimen are brownish black to black, with yellow to orange-yellow tips to the abundant guard hairs; these tips are narrow to broad and scattered very sparingly to profusely over the back, giving the animal a black appearance, with yellow-tipped guard hairs; those with most yellow are not unlike intermediate Yukon specimens; those with least yellow approach one Nova Scotia specimen, but are more orange yellow.

The Great Plains specimens (southern Alberta and Saskatchewan) show considerable variation; three are similar to the yellow-phased Yukon specimens, but somewhat more greenish-yellow; one is like the intermediate Yukon specimens, and one is similar to the Manitoba specimens.

Skull Characters

The skulls of females show smaller ridges and crests, and differences appear in a lesser degree than in those of males. From Ontario eastward the skulls of adult males have a median swelling in the frontals and a slight depression in the frontal parietal area; cranial ridges are rather evenly developed throughout their length, sagittal and occipital crests moderate; nasals are short, with broadly rounded or subtruncate posterior margin; the cranial ridges and sagittal crests form a *Y* with long, straight arms; a small lateral ridge runs diagonally down across the side of the premaxilla, from the base of the rostrum (Figs. 3*a*, 3*b*).

A single, incomplete Manitoba skull (female) has very short nasals, with broadly rounded posterior tips and has the ridge on the side of the rostrum.

A Wood Buffalo Park adult female exhibits some of the characters of eastern Canada skulls (though the skin is of a "yellow-haired porcupine") and some of those of the Yukon type.

Yukon Territory and Chitina River, Alaska, adult males have longer nasals (on the average) with more pointed posterior margins; cranial ridge unevenly developed, with more development over the orbit; less frontal swelling and more of a frontal-parietal depression and a slight tendency toward a shortening and an inward bowing of the arms of the *Y* formed by the cranial ridges and sagittal crest; crests moderate; no ridge on the side of the rostrum (Figs. 4*a*, 4*b*).

British Columbia specimens (especially from the north) have no frontal swelling; very deep frontal-parietal depression; cranial ridges not evenly developed, but exaggerated over the orbit; crests greatly developed; arms of the *Y* formed by the sagittal crest and cranial ridges short, and much bowed inward; nasals long, with the posterior margin more pointed; no ridge on the side of the rostrum; northern British Columbia specimens are rather uniform; those from southern part of the province are more variable, and differ less from Yukon skulls (Figs. 5*a*, 5*b*).

Great Plains adult skulls are those of females, and not significantly different from those of British Columbia females.

The following forms are recognizable in Canada.

GENUS *Erethizon* F. CUVIER (6, p. 432)

1822. Mém. Muséum hist. nat., Paris, 9 : 432. Type, *Hystrix dorsata* Linnaeus.

Erethizon dorsatum picinum Bangs

Labrador porcupine

1900. *Erethizon dorsatus picinus* Bangs. Proc. New England Zool. Club, 2 : 37. Type locality, L'Anse au Loup, Strait of Belle Isle, Labrador (3, p. 37).

1924. *Erethizon dorsatum picinum* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

Described as larger than *dorsatum*; tail a little shorter; colour plain black, without white-tipped hairs; skull rather larger; rostrum stouter, incisors broader and stronger, dull yellow instead of orange; molariform teeth smaller.

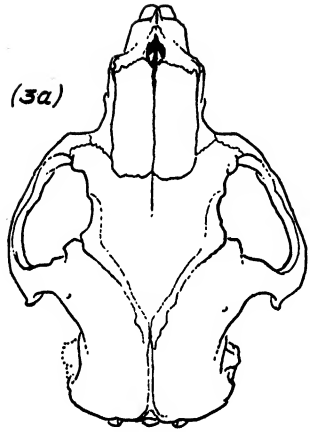
TABLE II

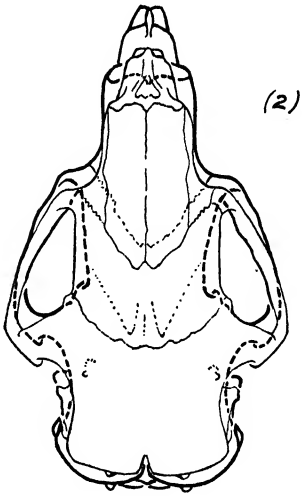
MEASUREMENTS OF *Erethizon dorsatum picinum* BANGS

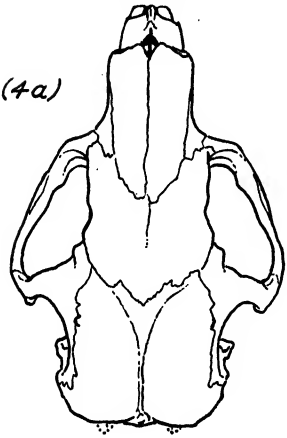
Topotypes	External measurements			Skull measurements			
	Total length	Tail vertebrae	Hind foot	Condylo-basal length	Zygomatic breadth	Least interorbital breadth	Length of nasals
From Strait of Belle Isle, Labrador							
Five old males							
8835 *M.C.Z.* (Bangs collection)	725	172	115	—	—	—	—
8838 *M.C.Z.* "	753	195	97	—	—	—	—
8837 *M.C.Z.* "	757	192	106	—	—	—	—
8834 *M.C.Z.* "	757	204	97	—	—	—	—
8839 *M.C.Z.* "	790	166	124	99.6	76	34	35.6
Average	756.4	185.8	107.8	—	—	—	—
Two old females							
8840 *M.C.Z.*	697	183	104	—	—	—	—
8836 *M.C.Z.*	762	182	112	—	—	—	—
From Chimo, Que.							
N.M.C. No. 11058	—	—	—	90	68	31	30.5

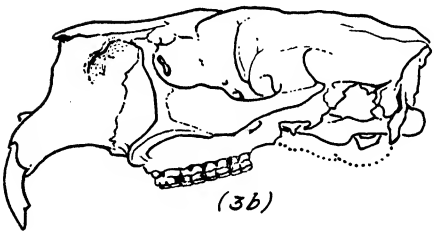
Measurements of topotypes as given by Bangs (3, p. 38).

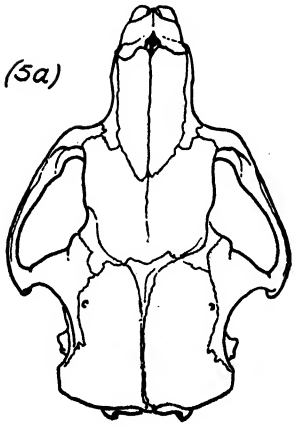
FIGS. 2 TO 4. *Skulls of Erethizon dorsatum.* FIG. 2. *E. dorsatum nigrescens, outlines of two skulls of adult males from Yahk, B.C. to show variation.* FIG. 3*a*. *E. dorsatum dorsatum, adult male, Gaspé, Que.* FIG. 3*b*. *E. dorsatum dorsatum, side view of 3a.* FIG. 4*a*. *E. dorsatum myops, young adult male, Teslin Lake, Yukon Territory.* FIG. 4*b*. *Side view of 4a.* FIG. 5*a*. *E. dorsatum nigrescens, adult male, Hot Springs, Dean Channel, B.C.* FIG. 5*b*. *Side view of 5a.*

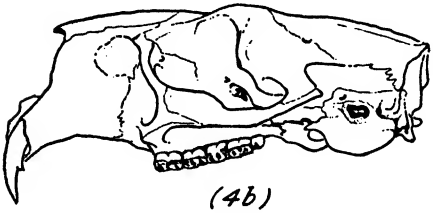

(3a)

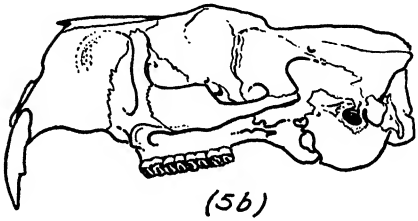

(2)


(4a)


(3b)


(5a)


(4b)


(5b)

Range

Common and generally distributed in Labrador and northeastern Quebec, from the St. Lawrence north to the semibarrens, west to Chimo but otherwise its western limits are unknown, though porcupines undoubtedly occur here and there over Ungava Peninsula south of the tundra.

Remarks

Lack of material prevents evaluation of this form. A single Chimo skull in the National Museum collection, compared with *dorsatum*, has the characters assigned to *picinum*. A single albino skin without skull, taken near Chimo, was received at the National Museum June 29, 1943, but gives no clue to the normal colour of pelage.

Specimens Examined

Two[1]. Quebec: Fort Chimo; two (one skin and one skull, from different animals).

Erethizon dorsatum dorsatum (Linnaeus)

Canada porcupine

1758. [*Hystrix*] *dorsata* Linnaeus. Systema naturae, ed. 10, 1 : 57 (10, p. 57). Type locality, Eastern Canada.

1822. *E*[*rethizon*] *dorsatum* F. Cuvier. Mém. Muséum hist. nat., Paris, 9 : 432 (6, p. 432).

1924. *Erethizon dorsatum dorsatum* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

Characterized by the small amount of yellowish or whitish tipping of the guard hairs, in most specimens confined to the nape, hip region, and tail; by little or no white on the head; by the pale yellowish-white colour of these tips; by the intense black or brownish black colour of the dark pigmented fur; by the slight swelling of the skull in the frontal region between the orbits, and the slight depression behind it; by the moderately developed cranial ridges running back in a straight line to fuse and form a short sagittal crest; by the nasals being short, subtruncate on their posterior end, or broadly rounded; by the presence of a ridge on the side of the rostrum on the premaxilla, running down diagonally from the base of the rostrum (Figs. 3*a*, 3*b*).

Range

Northeastern United States and Eastern Canada, from Nova Scotia and Gaspé to Manitoba and northern Saskatchewan; animals intergrading with *myops* in colour in the southern Yukon, and in skull characters in the Wood Buffalo Park, northern Alberta, according to our material.

There is considerable variation in the pelage of our 12 Nova Scotia, New Brunswick and Gaspé skins and nine Ontario and western Quebec skins. The specimens showing the least yellowish white have the ground colour black, or slightly brownish black, with small yellowish-white tips to a few of the guard hairs of the nape, the hips, and many of those fringing the tail. Eight

[1] *All specimens indicated as examined in the course of this study are in the mammal collection of the National Museum of Canada unless otherwise specified.*

TABLE III

MEASUREMENTS OF *Erethizon dorsatum dorsatum* (LINNAEUS)

	External measurements			Skull measurements				
	Total length	Tail vertebrae	Hind foot	Condylo-basal length	Zygomatic breadth	Length of nasals	Least interorbital breadth	Width of rostrum
Adult males								
Nova Scotia	800	210	110					
	830	230	100	101	72	31	33	23
Gaspé	788	181	108	103	—	33	31.5	26
Ontario	725	262	111	105	73.5	33	32	23
	737	230	108	98	—	30	32	24.5
Adult females								
Western Quebec	—	—	—	85	68	31	—	22.5
	—	—	—	—	—	34.5	—	23.5
Ontario	734	229	86	94	70	32	30	22.5
Manitoba, Thicket Portage	673	148	93	—	—	27.5	—	21.5
Adult, sex?								
New Brunswick	648	175	96	—	—	30	—	—
Western Quebec	—	—	—	91	—	31	33	23
				94	66	30.5	29.5	23
				99	—	33	29.5	20.5
				101	71	33.5	31	25
				89	—	28.5	31	—
				—	—	31.5	—	22
				99	—	35.5	34.25	23.5

of the Maritime specimens and six of the Ontario–western-Quebec skins differ slightly in having more yellowish-white–tipped guard hairs, with sometimes only a few scattered sparingly over the back. One Nova Scotia specimen has many of the guard hairs of the back narrowly tipped with yellowish white and with considerably more white showing in the spines of the head; two Ontario specimens have whitish-tipped guard hairs through most of the dorsal pelage, and one has them conspicuous over the whole back, flanks, and legs.

Two northwest Manitoba specimens differ somewhat in having the general tone slightly more brownish black; both have yellowish-white–tipped guard hairs scattered over the upper part of the body, in one of them most of the guard hairs are light-tipped. A skull from Sandilands Forest Reserve, southeast Manitoba, collected by J. Dewey Soper is definitely of this form, though it is that of a subadult female. Mr. Soper supplied us with a note that the species is common in Prince Albert National Park (north of Prince Albert), Sask., and that two specimens he saw there were typical *dorsatum*, being very dark, practically black.

There is variation in the skulls, but it is less than in skulls from southern British Columbia.

Specimens Examined

Nova Scotia: Shelburne Co., Barrington Passage, one; Guysborough Co., East Roman Valley, five.

New Brunswick: Gloucester Co. near Bathurst, three.

Quebec: Gaspé, one; Pontiac Co., 14.

Ontario: near Ottawa, one; Petawawa, one; Lanark Co., one; Lennox Co., one; Pancake Bay (Algoma District), one; Thunder Bay, one; York Factory, one (fragmentary skull only).

Manitoba: Thicket Portage (Hudson Bay Railway, mile 165), one; Herchmer, mile 412, one; Sandilands Forest Reserve, one[1].

Total, 34 specimens.

Erethizon dorsatum myops Merriam

Alaska porcupine

1900. *Erethizon epixanthus myops* Merriam. Proc. Wash. Acad. Sci. 2 : 27 (12, p. 27). Type locality, Portage Bay, Alaska Peninsula, Alaska.

1924. *Erethizon epixanthum myops* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

Compared with *dorsatum* our specimens are characterized by the average browner colour of the dark pigmented fur; by the more abundant guard hairs; by the much greater amount of yellow in the guard hairs, by the distinctly rusty yellow tinge of this pale fur; by the narrower rostrum; by the longer nasals, more pointed posteriorly; by lack of swelling in the frontals; by a deeper depression in the frontal parietal area; by the cranial ridges being more developed over the orbit, moderately developed posteriorly; by moderate sagittal and occipital crests, and by the lack of a ridge on the side of the rostrum (Figs. 4*a*, 4*b*).

Range

Northern Alberta (Wood Buffalo Park) and southern Yukon to Alaska.

Remarks

We have no topotypical *myops* and refer the southern Yukon specimens tentatively to that form. Certainly they are different enough from our British Columbia series to represent another race; in the matter of outline of zygomatic arches, there is little more than a tendency toward the bowed conditions as described for *myops*.

A Wood Buffalo Park specimen is rather blackish generally, with abundant guard hairs, most of which are moderately tipped with rusty yellow; in appearance it is a black animal with yellow-tipped fur.

We have a series of 16 skins from southern Yukon, mostly from near Teslin Lake. Four of them have blackish or dark brownish general pigmentation with rather sparse guard hairs, two have only a few of the guard hairs on the nape and in the tail yellow-tipped; one has narrowly yellow-tipped guard

[1] *Kindly loaned by J. Dewey Soper of Winnipeg, Man.*

TABLE IV

MEASUREMENTS OF *Erethizon dorsatum myops* MERRIAM

	External measurements			Skull measurements				
	Total length	Tail vertebrae	Hind foot	Condylo-basal length	Zygomatic breadth	Length of nasals	Least inter-orbital breadth	Width of rostrum
Two adult males, Teslin Lake, Y.T. area				100	71.5	38	—	23
				96	—	40	—	21
Two adult females (?), Teslin Lake, Y.T.				104	70	40	28.5	21.5
				95	—	38	28	20
Adult female, Wood Buffalo Park, Alta.				93	73	31.5	28.5	21.5
Four adult males, Chitina River, Alaska	800	220	114	104	—	46.5	28.5	22
	750	170	97	99	70	36	29	22.25
	—	—	—	97	70	37	27	21.75
	—	—	—	107	71.5	38.75	31	24.5

hairs sparingly scattered over the back, and one has them fairly plentiful; they are very similar to eastern porcupines, differing only in the yellow averaging more rusty; on pelage alone one is indistinguishable from a Nova Scotia specimen even in the tint of the yellow.

Six other Yukon specimens are very different from the above; the dark pigmented fur is brownish and the extensive yellow tips of the profuse guard hairs are profusely distributed over the body, averaging rusty yellow in colour; the yellowish colour extends onto the hind part of the head. This gives the animal a distinctive brownish-yellow general appearance. In these six specimens four have the under surface of the tail mostly yellow, mixed with a little brownish; one has it mostly brownish; and one has it blackish. One Yukon specimen is blackish with abundant yellow-tipped guard hairs; the remaining five specimens are variably intermediate between the black and the yellow phases described above; the two Manitoba specimens would fit into this part of the series, except for having somewhat more whitish, rather than yellowish tips to the guard hairs.

Two Chitina glacier skins differ considerably from each other; one is pale brownish generally, with abundant yellow guard hairs, the yellowest porcupine of our collection; the other is like the intermediate Yukon specimens.

The above demonstrates that in the Teslin Lake area of Yukon Territory porcupines with eastern type and with western type pelage occur, and these are connected by intermediates. The western type of pelage occurs in a pronounced form as far eastward as Wood Buffalo Park.

In comparing the skulls, those from Teslin Lake (of which about eight are suitable for comparison) are all fairly distinct from eastern skulls on the characters given above.

However the skull of the Wood Buffalo Park specimen described above is especially illuminating in this connection. It is of the eastern porcupine type, in the short nasals, though they are rather pointed posteriorly, and in the swelling of the frontals; in the shape of the zygomatic arches it is of the *myops* type, the arches being more bowed. Though it is definitely *myops* in skin characters, in skull it shows a pronounced approach to *dorsatum*.

Specimens Examined

Alberta: Wood Buffalo Park, one.

Yukon Territory: vicinity of Teslin Lake, 15; Parent Creek, Duncan District, one; Whitehorse, one.

Alaska: Chitina River, four.

Total, 22 specimens.

Erethizon dorsatum nigrescens Allen

Dusky porcupine

1903. *Erethizon epixanthus nigrescens* Allen. Bull. Am. Museum Nat. Hist. 9 : 558. Type locality, Shesley River (45 miles from Telegraph Creek, B.C.) (2, p. 558).

1924. *Erethizon epixanthum nigrescens* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

The dark pigmented fur is blackish, as in *dorsatum*, from which it is distinguished by the more abundant guard hairs, with light tips averaging considerably more plentiful, scattered over the back; by the average orange-yellow rather than yellowish-whitish colour of these light tips; compared with our specimens of *myops* the general colour averages blackish, not brownish; the yellow tipping of the guard hairs is less extensive; and the colour of this tipping averages orange-yellow, rather than rusty yellow.

In the skulls, those from north British Columbia are not especially variable (Figs. 5*a*, 5*b*); those from south British Columbia are more so (Fig. 2). In some ways they represent a further divergence from the *dorsatum* skull than do *myops* skulls, from which latter they are distinguishable on the average by the greater development of the interior portion of the cranial ridge over the orbit giving a deeper frontal depression; by the moderately developed posterior portion of the cranial ridges curving together quickly, fusing together little back of the posterior margin of the frontals (instead of carrying far back, separately, and more nearly straight before meeting) giving a longer sagittal crest that is more developed; occipital crest higher; nasals long with more or less acute posterior end as in *myops*.

Range

British Columbia and Western Alberta, from Telegraph Creek and Jasper southward. Hall (8, p. 380), finds no difference between the skulls of British

TABLE V

MEASUREMENTS OF *Erethizon dorsatum nigrescens* ALLEN

	External measurements			Skull measurements				
	Total length	Tail vertebrae	Hind foot	Basal length	Zygomatic breadth	Length of nasals	Interorbital breadth	Width of rostrum
Yahk, B.C., adult males	802	240	116	103	72.5	39	28	21.5
	833	237	124	101	75	42.5	36.5	22.5
	860	278	116	118	—	44	28.5	22.5
Chilcotin, B.C., adult male				112	76	37.5	32.5	24
Rossland, B.C., adult male	860	300	115	106.5	73	39	31	23
Bella Coola area, B.C., adult males	790	240	106	103.5	71.5	40	29.5	22
	850	200	110	100	72.5	36	31.5	21.5
	760	245	110	105	70.5	36	29.25	24.75
	850	270	111	100	77.5	36	32.5	24.5
				105	—	40	33	24.5
				106	75.5	38	31	24
Bella Coola area, B.C., adult females	740	210	101	95	68.5	35.5	28.5	20.5
	830	240	94	95	68	35.5	30	22
Okanagan, B.C., adult females	825	240	105	102	72.5	45.5	33.75	24.5
Waterton Park Lake, Alta., adult female				98	70	39	27.5	17

Columbia and of California taken porcupines, and separates the former as *nigrescens* on the basis of the orange-yellow colour of the bands on the long guard hairs. Our specimens in the average also have orange-yellow tips to the guard hairs.

Hall says also that in summer pelage the long hairs all over the back are broadly tipped with yellowish, while in winter the long hairs are light tipped mainly over the neck region and the sides of the tail, lacking over the midback and most of the rest of the upper parts.

However, our material shows this is individual variation, and the chief difference between winter and summer pelage is that in winter the under fur is long, concealing the spines, while in summer the spines are more or less exposed. The latter condition naturally exposes some of the white of the quills.

Remarks

Twelve of our 14 British Columbia skins agree in being generally blackish rather than brownish in ground colour and in having the yellow tipping of the long, abundant guard hairs more or less orange-yellow.

The amount of this yellow tipping varies considerably, from sparse narrow bands on some of the guard hairs to wide conspicuous bands on most of the guard hairs. The general appearance is of a black animal, with yellow-tipped guard hairs. Hall (8, p. 380) suggests that winter specimens are blacker. We have February and July specimens, and while the short under fur in the summer specimens allows more of the spines to be visible and more of the white of them to be seen (instead of being completely concealed) it does not change the appearance of the animal otherwise.

Five of the above specimens have the under surface of the tail black, with only a tinge of brownish at the base; one has it mostly yellowish, and the others are intermediate.

In respect to general blackness, and in the amount of yellow tipping to the guard hairs these 12 British Columbia specimens average about the same as the extreme eastern specimens (one from Ontario and one from Nova Scotia). However the yellow averages considerably deeper and more orange.

Two specimens, one from the Bella Coola area and one from Hagensborg are more brownish generally, with whitish extending onto the head, and with more yellow in the guard hairs giving the appearance of yellow-brown animals that are not unlike some of the intermediate Yukon specimens. Two skins from south British Columbia are less orange yellow, perhaps tending toward *epixanthum*; but one Yahk specimen has the yellow tips to the guard hairs light orange-yellow.

In regard to the skulls, the Bella Coola series are rather uniform—in their deep frontal depression; the projection over the orbit; high sagittal and occipital crests; and long sagittal crest; with the cranial ridges sharply pinched together. This is a further modification away from *dorsatum* of some of the characters seen in the south Yukon series (*myops*). However, those from elsewhere in British Columbia are more variable. Especially is this true of three skulls from near Yahk, in which the basal length, interorbital width, length and width of rostrum, the angle of projection of the incisors, and the height of the occipital crests vary greatly (Fig. 2). The characters of the skins from Rossland and Yahk however place them with this form. The Waterton Lake specimen is represented by a skull only, and is provisionally referred here. Davis (7, p. 344) refers specimens from southern Idaho to *epixanthum*, and Waterton Lakes Park specimens might be expected to be this form. According to Hall (8) the skulls of the two are indistinguishable.

Specimens Examined

Alberta: Jasper, one; Waterton Lakes Park, one (skull).

British Columbia: Yahk, three; Chilcotin, one; Rossland, one; Okanagan, one; Mackenzie Pass, Rainbow Mountains, one; Mount Brilliant, Rainbow Mountains, one; Dean Channel, six.

Total, 18 specimens.

Erethizon dorsatum epixanthum Brandt
California porcupine

1835. *Erethizon epixanthus* Brandt. Mém. acad. St-Pétersbourg (Sér. 6), 3 (Sci. nat. 1) : 390 (5, p. 390). Type locality, California (9, p. 27).
1924. *Erethizon epixanthum epixanthum* Miller. U.S. Natl. Museum, Bull. 128 : 437 (13, p. 437).

Diagnosis

Great Plains specimens differ from any of our series of *myops* and *nigrescens* in the more greenish yellow, less orange or rusty yellow of the light tipping of the guard hairs. The available adult skulls are of females and, from those of our specimens of *nigrescens*, differ chiefly in the cranial ridges being straight and meeting farther back on the skull; however females generally show less differences than do males, and we have fewer skulls of females to show the range of variation.

The variations found in both external characters and skull characters in the Great Plains region may be due to the fact that this large area has little country suitable for porcupines, and small inbred groups in this area of intergradation may be indicated.

Two of the adult skins from western escarpment of Cypress Hills (Lodge Creek, Alta.) are very similar to some of the Yukon specimens referred to *myops* but the general tone of the yellow of guard hairs is greenish yellow rather than rusty yellow; one of these specimens has the under surface of the tail mostly yellow, mixed with a little brown, and in the other the tail is mostly brownish black. A smaller subadult specimen from the same locality is blackish brown with conspicuous whitish tips to the guard hairs on nape and rump and a few on the back, similar to the Manitoba specimens, but all the Lodge Creek specimens have the typical long, narrow nasals of the western porcupines.

Two adult female skins from Lonesome Butte, Sask., about 200 miles farther east on the Great Plains, compared with Lodge Creek specimens, are much paler in general appearance, with heavier growth of pale greenish yellow guard hairs from head to tail and with face and throat noticeably lighter

TABLE VI

MEASUREMENTS OF *Erethizon epixanthum epixanthum* BRANDT

	External measurements			Skull measurements				
	Total length	Tail	Hind foot	Condylo-basal length	Zygo-matic breadth	Nasal length	Inter-orbital width	Width of rostrum
Lodge Creek, Alta., adult females	790 765	220 205	120 100	98 96	71.5 69	43 37.5	34 39	26 22

gray in colour; the skulls also approach the eastern porcupine in comparatively greater breadth and shortness; nasals shorter, one specimen (No. 8320) having broad, short nasals only 30 mm. long.

Range

From western United States into at least the Cypress Hills area of southeastern Alberta and southwestern Saskatchewan, Canada.

Remarks

Swenk (17, pp. 115–125) described *bruneri* from Nebraska, the type from Mitchell, Scottsbluff County, Neb., and paratype from near Mullen, Cherry County, Neb.; he referred one specimen from Kansas, three from Wyoming, one from eastern Montana to the same form on the basis of recorded skull measurements. Amongst other characters it is supposed to be larger than either *epixanthum* or *nigrescens*. Our specimens compared with British Columbia material show no appreciable difference in size, and on the basis of shade of yellow are referred to *epixanthum*, though we have no topotypical *epixanthum* nor *bruneri* for comparison. Davis (7, p. 344) records specimens from southern Idaho as *epixanthum*.

"Yellow-haired" porcupines have been recorded taken as far east as the Turtle Hills, N.D., and might be expected in southern Manitoba where porcupines are known to occur; specimens from that area would be especially interesting.

Specimens Examined

Alberta: Lodge Creek, near western edge of Cypress Hills, three.

Saskatchewan: Lonesome Butte, southwest of Wood Mountain, near Montana boundary, two.

Total, five specimens.

References

1. ALLEN, J. A. Monographs of North American Rodentia. Rept. U.S. Geol. Survey Terr. 11 : 379-398. 1877.
2. ALLEN, J. A. Mammals collected in Alaska and northern British Columbia by the Andrew J. Stone expedition of 1902. Bull. Am. Museum Nat. Hist. 19 : 521-567. 1903.
3. BANGS, O. Three new rodents from southern Labrador. Proc. New England Zool. Club, 2 : 35-41. 1900.
4. BANGS, O. List of the mammals of Labrador. *In* Grenfell, W.T. *et al.*, Labrador, the country and the people. The Macmillan Company, New York. 1900.
5. BRANDT, J. F. v. Mammalium rodentium exoticorum novarum vel minus rite cognitorum. Mus. Acad. Zool. descriptiones et icones. (1834). Mém. acad. St-Pétersbourg (Sér. 6), 3 (Sci. nat. 1) : 357-442. 1835.
6. CUVIER, F. Mém. Muséum hist. nat., Paris, 9 : 432. 1822.
7. DAVIS, W. B. The recent mammals of Idaho. The Caxton Printers, Caldwell, Idaho. 1939.
8. HALL, E. R. Mammals collected by T. T. and E. B. McCabe in the Bowron Lake region of British Columbia. Univ. Calif. Pub. Zool. 40 : 363-386. 1934.
9. HOLLISTER, N. Mammals of the Alpine Club expedition to the Mount Robson region. Can. Alpine J., Spec. No., 1-44. 1912.

10. LINNAEUS, C. v. Caroli Linnaei Systema Naturae per Regna tria naturae, Secundum Classes, Ordines, Genera, Species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio Decima, Reformata. Holmiae, Impensis Direct, Laurentii Salvii. 1758.

11. MEARNS, E. A. Descriptions of six new mammals from North America. Proc. U.S. Natl. Museum, 19 : 719-724. 1897.

12. MERRIAM, C. H. Descriptions of twenty-six new mammals from Alaska and British North America, 13-20. Papers from the Harriman Alaska expedition. Proc. Wash. Acad. Sci. 2 : 1-694. 1900.

13. MILLER, G. S., JR. List of North American recent mammals, 1923. U.S. Natl. Museum, Bull. 128. 1924.

14. SCHULTZ, A. H. Bregmatic fontanelle bones in mammals. J. Mammal. 4 : 65-77. 1923.

15. SWARTH, H. S. Birds and mammals of the Skeena River region of northern British Columbia. Univ. Calif. Pub. Zool. 24 : 315-394. 1924.

16. SWARTH, H. S. Mammals of the Atlin region, northwestern British Columbia. J. Mammal. 17 : 398-405. 1936.

17. SWENK, M. H. On a new subspecies of porcupine from Nebraska. Univ. Nebraska Studies, 16 : 115-125. 1916.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. OCTOBER, 1943 NUMBER 10

THE CARDIAC ACTION OF POSTERIOR PITUITARY EXTRACT IN PHYSIOLOGICAL DOSES, IN THE NORMAL DOG, AND AFTER PARTIAL AND COMPLETE DENERVATION OF THE HEART[1]

BY MARGARET E. MACK. SAWYER[2] AND G. H. ETTINGER[3]

Abstract

In the normal dog continuous infusion of dilute posterior pituitary extract produces a maximal inhibition of the heart, i.e. slowing to about one-half of the resting rate, with usually a rise in blood pressure of 10 to 30 mm. of mercury.

After bilateral thoracic sympathectomy, posterior pituitary extract also produces maximal inhibition. This inhibition, like that produced in the normal dog, is abolished by atropine.

After bilateral vagotomy posterior pituitary extract produces a moderate but not maximal inhibition. This inhibition is not abolished by atropine.

After bilateral thoracic sympathectomy and unilateral vagotomy, posterior pituitary extract produces a maximal effect.

After total denervation of the heart, posterior pituitary extract produces no inhibition of the heart and the rate is unchanged.

Characteristic changes are produced by posterior pituitary extract in the electrocardiogram of normal dogs. After total denervation no change takes place.

It is concluded that the slowing of the dog's heart that is produced by continuous intravenous infusion of posterior pituitary extract is entirely due to its action through the inhibitory fibres of the vagus and sympathetic nerves.

The bradycardia produced in the dog by posterior pituitary extract, according to Resnik and Geiling (11), and Gruber and Kountz (4), is due to a two-fold action: (*a*) a reflex vagal slowing, and (*b*) a direct depressant action on the myocardium, possibly through coronary arterial constriction. Melville (7) considers that there is no direct myocardial depression; all changes apart from vagal slowing are caused by constriction of the coronary arteries. In the cat, Bacq and Dworkin (1) obtained results that indicated that the slowing produced by pitressin was entirely due to an action upon the extra-cardiac connections of the cardiac nerves.

In order to re-explore the site and nature of the inhibitory process, we determined the action of posterior pituitary extract in the normal dog, and in the dog after partial and complete cardiac denervation. These experiments were done in the course of an investigation of the effects of chronic

[1] *Manuscript received June 15, 1943.*
Contribution from the Department of Physiology, Queen's University, Kingston, Ont. With financial assistance from the National Research Council of Canada.

[2] *Fellow in Physiology.*

[3] *Professor of Physiology.*

excitation of the inhibitory mechanism of the dog's heart, by infusion of a dilute solution of posterior pituitary extract for two hours daily, over a period of four and one-half years.

Previous investigators tested the action of posterior pituitary extracts, including pitressin, by injecting large single doses of commercial or other concentrated preparations; these doses are more liable to be toxic than physiological. The results were often complicated by the use of anaesthesia. In our experiments a more nearly physiological method of administration was used. Posterior pituitary extract was slowly administered in dilute solution by continuous infusion for 30 to 120 min. to trained, unanaesthetized dogs weighing from 12 to 19 kg., at such a rate as to produce a continuous maximal bradycardia without disturbing effects such as nausea or vomiting. This required only about two to two and one-half pressor units per hour.

Method

Dogs were trained to lie quietly on a table, until their resting-heart rates could be obtained accurately before each infusion was started. After application of alcohol to the leg, a sterile hypodermic needle was inserted into a leg vein and taped in place. This was connected to an outlet on the bottom of an elevated sterile flask containing the infusate by a sterile rubber tube, on the course of which was an air trap by which the rate of flow could be determined in drops. Infusion by gravity flow was continued for 30 to 120 min. and the heart rate counted every 5 to 10 min. Occasionally a continuous count was made for five minutes after the start of the infusion to obtain any immediate changes. After a few trials the dogs remained quiet throughout the infusion, which usually seemed to have a sedative effect.

TABLE I

THE EFFECT OF INFUSION OF POSTERIOR PITUITARY EXTRACT ON THE HEART RATE OF THE DOG, NORMAL, AND WITH CARDIAC SYMPATHECTOMY, REPEATED DAILY OVER A PERIOD OF YEARS

Dog	Condition	Resting rate per minute	Minimal infusion rate, per min.	Resting rate per minute	Minimal infusion rate, per min.
		1938		1942	
Sandy	Normal	64	30	60	44
Toy	Normal	66	40	80	44
Scarface	Normal	68	30	76	36
Rufus	Normal	72	40	88	44
		1940		1942	
General	Cardiac sympathectomy	64	36	72	44
Sargent	Cardiac sympathectomy	68	44	68	40
Ted	Cardiac sympathectomy	72	40	76	44

The pituitary extract used was prepared by the Connaught Laboratories, Toronto, Ont. It contained 10 pressor units per cc., and had tricresol, 0.1% as a preservative. A solution was made of 1 cc. of the extract dissolved in 500 cc. of sterile normal saline. Each animal received as much as 250 cc. of this dilute solution. Daily infusion for periods up to four and one-half years produced little evidence of a tolerance (see Table I).

The effect of atropine (0.2 to 0.3 mg./kg.), administered intravenously before infusion and during infusion, was determined in all dogs after repeated experiments had demonstrated the normal response to posterior pituitary extract.

The effect of the infusion on blood pressure was determined by connecting a mercury manometer to a sterile needle or arterial cannula inserted into the femoral artery under local anaesthesia.

Cardiac denervation was done after the normal response to posterior pituitary infusion had been recorded. The response to infusion and the effect of atropine on the response were determined after operative recovery from each step in the denervation. The sympathetic nerve supply to the heart was interrupted by aseptically removing the stellate ganglia and the thoracic sympathetic chains down to the ninth rib, in two steps, after the method of Cannon *et al.* (2). Cervical vagotomy was done in two stages by aseptic removal of 3 cm. of each of the nerves. At least six days were allowed to elapse between operations. In most cases a period of several weeks passed. Complete denervation of the heart was done in three stages: first, right thoracic sympathectomy and right cervical vagotomy; second, left thoracic sympathectomy; third, left cervical vagotomy. Excision of the vocal cords at the time of the first stage prevented any later difficulty in respiration. Completely vagotomized dogs retained little food, but were maintained for periods of 10 days to two months by supplementing feeding with intravenous infusion of glucose saline.

Electrocardiograms were taken on all animals at all stages of the experiment.

Results

A. Normal Animals

The Effect of Posterior Pituitary Infusion on the Heart Rate

The resting-heart rates in the trained dogs were from 60 to 90 per minute. Continuous infusion with posterior pituitary extract invariably inhibited the heart to about half the resting rate (Table I). This inhibition commenced about one to two minutes after the start of infusion, became maximal in 5 to 10 min. and was maintained thereafter as long as the infusion continued. There was sometimes a very brief period of acceleration in the first minute immediately after the start of infusion, before the heart began to slow. (This

acceleration was always observed by Gruber and Kountz (4) and by Resnik and Geiling (11) who used large single injections of extract.) After the infusion was stopped the heart rate gradually increased and came back to the resting rate in 20 to 30 min. Figs. 1 and 2 show characteristic

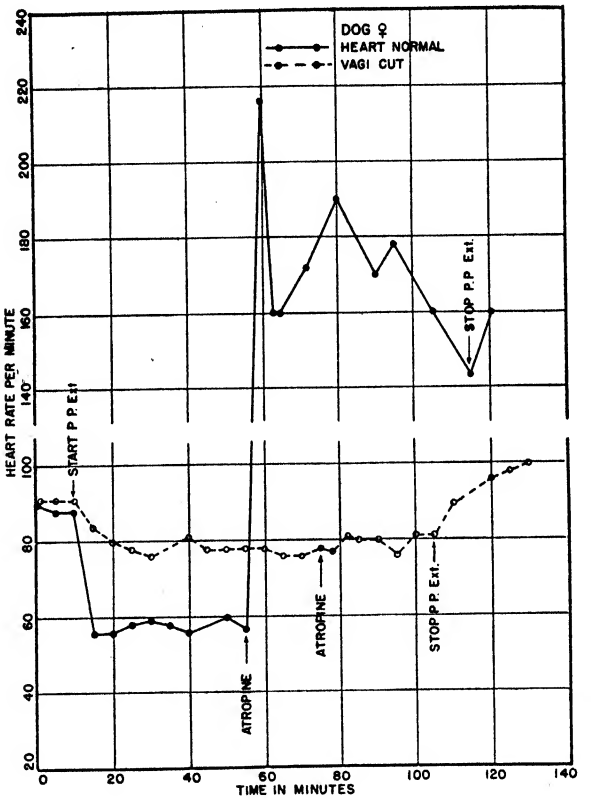


FIG. 1. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the heart rate of a normal, unanaesthetized dog before and after section of the vagi.*

changes in heart rates in normal dogs during infusion with posterior pituitary extract.

The Effect of Atropine on the Response to Posterior Pituitary Infusion

The effect of atropine varied according to whether it was administered before the infusion had started, or during the infusion. Given without infusion it produced a tachycardia up to 200/min. within five minutes, followed by a slow and steady decline in rate, with a return to the normal in one and one-half to two hours. When pituitary extract infusion was started 10 to 30

min. after the atropine injection, it usually produced no inhibition. The heart either maintained the atropine effect with its gradually declining influence, or there was a brief period of further acceleration (Fig. 3). This latter response is similar to that reported by Resnik and Geiling (11) following a

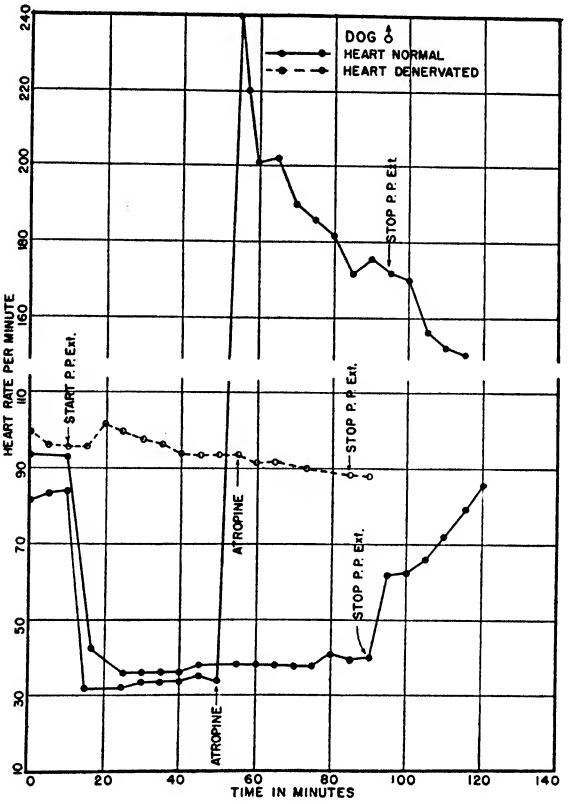


FIG. 2. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the heart rate of a normal, unanaesthetized dog before and after complete denervation of the heart.*

single injection of posterior pituitary extract. Gruber and Kountz (4) found that pitressin slightly slowed the atropinized heart.

When atropine was administered during infusion with posterior pituitary extract, and while the bradycardia was maximal, it invariably produced an intense tachycardia within two minutes (Figs. 1 and 2). The heart rate was higher than that producible in the same dog by atropine alone. For example, in a dog in which infusion of posterior pituitary extract normally slowed the

heart rate from 62 to 34 per minute, and in which atropine without infusion raised the heart rate to 210 per minute, atropine during infusion raised the rate to 244 per minute.

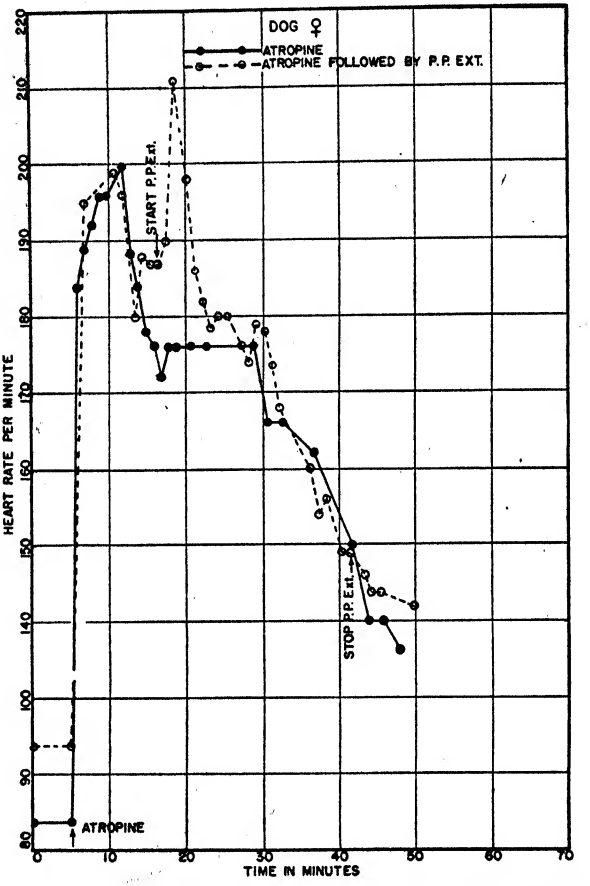


FIG. 3. *The effect of atropine, and atropine followed by infusion of posterior pituitary extract, on the heart rate of a normal, unanaesthetized dog.*

It is clear that atropine prevents the bradycardia produced by posterior pituitary infusion.

Electrocardiographic Changes Produced by Posterior Pituitary Infusion

A number of investigators have studied the effect of single injections of posterior pituitary extract and pitressin on the electrocardiogram. A list of references may be found in a paper by Melville (7). Electrocardiograms taken during continuous infusion showed many changes similar to those reported

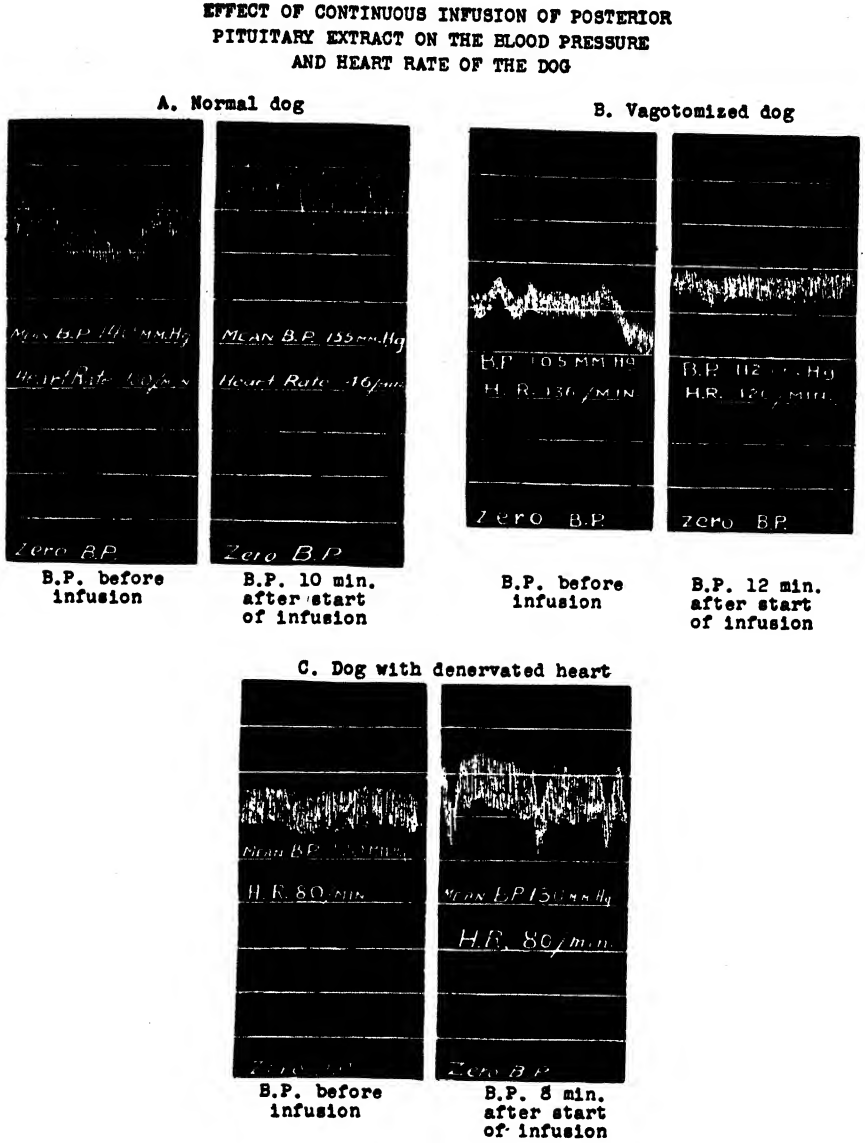


FIG. 4. *Change in femoral arterial blood pressure produced by infusion of posterior pituitary extract under local anaesthesia in the dog, normal, after vagotomy, and after complete denervation of the heart.*

after single injections (Figs. 5 and 6). Within two minutes of the start of infusion a marked slowing in rate took place, sometimes accompanied by an increased *P–R* interval. Changes in the *T* wave were most frequently

observed. Often within 30 sec. of the start of the infusion it became much more prominent, its amplitude increased and its shape peaked. A *T* wave inverted or diphasic before infusion might become upright and more prominent during infusion. Changes in the *P* wave were not common. Occasionally it became slightly notched. Sometimes heart block occurred. Grouping of beats was occasionally seen after infusion had gone on for some time; pulsus bigeminus was the most common of these.

The tachycardia produced in the normal dog by atropine was accompanied by a shortened *P–R* interval, without disturbance of the *T* wave. Infusion of posterior pituitary extract after atropine produced, within one minute, the characteristic heightening of the *T* wave, but no other changes. Atropine injected during the infusion produced a lengthened *P–R* interval, heart block, irregularity with pulsus bigeminus and trigeminus, all within the first half-minute. The *T* wave remained high. After this brief irregularity a marked regular acceleration occurred, with shortened *P–R* interval, the *P* and *T* waves frequently superimposed (Figs. 5 and 6). Gruber and Kountz (4) noted similar effects of atropine given two to three minutes after a single large dose of pitressin.

The Effect of Posterior Pituitary Infusion on Blood Pressure

A single large dose of pitressin or pituitary extract in the unanaesthetized dog produces (3, 4, 6, 7, 14) a sharp fall in blood pressure, preceded by a slight rise and followed by a second rise. A smaller dose may produce a pure

TABLE II

CHANGES IN HEART RATE AND BLOOD PRESSURE PRODUCED IN THE DOG UNDER LOCAL ANAESTHESIA BY INFUSION WITH POSTERIOR PITUITARY EXTRACT

Dog	Condition	*Resting heart rate per min.	Minimal infusion heart rate per min.	Fall in heart rate per min.	Resting blood pressure, mm. Hg.	Infusion blood pressure, mm. Hg.	Change, mm. Hg.
Barney	Normal	100	46	54	140	165	+25
Gunner	Normal	90	64	26	140	150	+10
Major	Normal	114	80	34	170	180	+10
Mike	Normal	72	52	20	130	125	− 5
General	Cardiac sympathectomy	120	76	44	140	160	+20
Ted	Cardiac sympathectomy	88	60	28	140	160	+20
Sargent	Cardiac sympathectomy	100	76	24	140	170	+30
Sally	Vagotomized	136	105	31	105	112	+ 7
Sambo	Vagotomized	148	132	16	110	120	+10
Katy	Vagotomized	120	110	10	115	135	+20
Colonel	Total cardiac denervation	80	80	0	120	135	+15

* *The slight restlessness of the animals under the conditions of the experiment raised the resting rate and prevented maximal inhibition.*

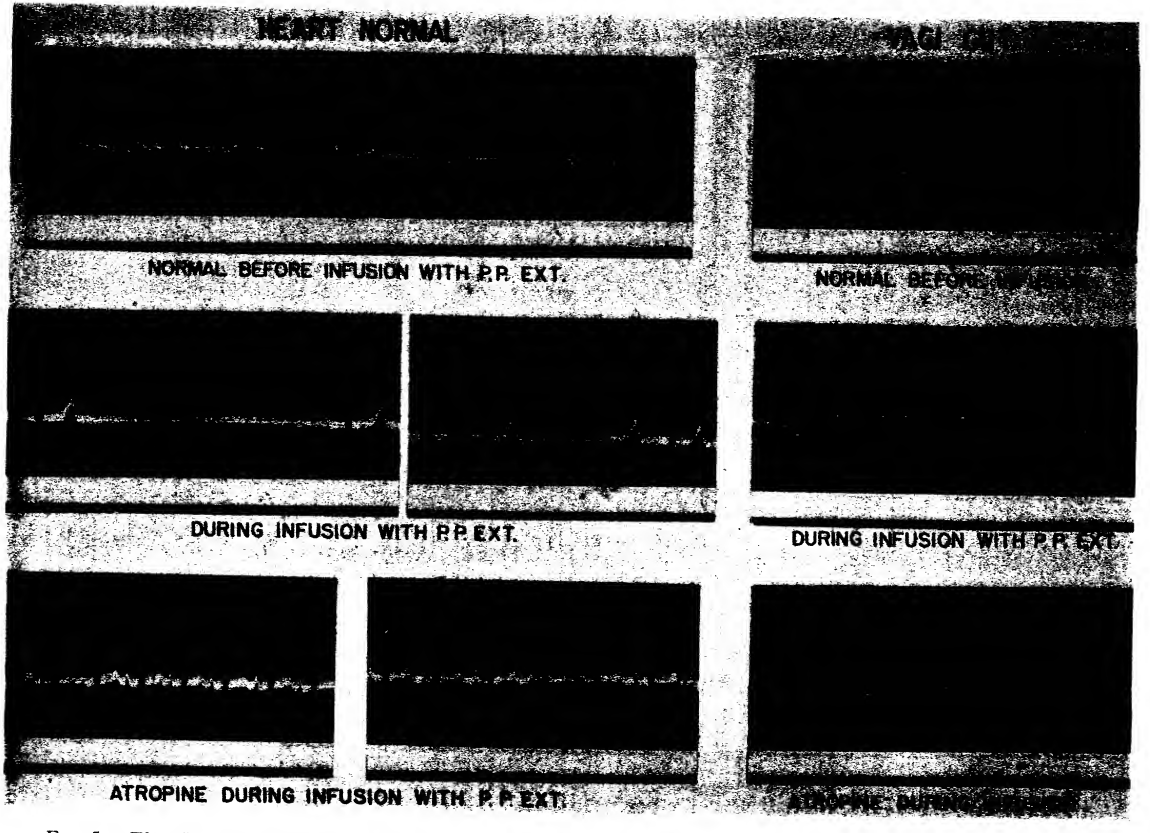


FIG. 5. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the electrocardiogram of a dog before and after section of the vagi (Lead II).*

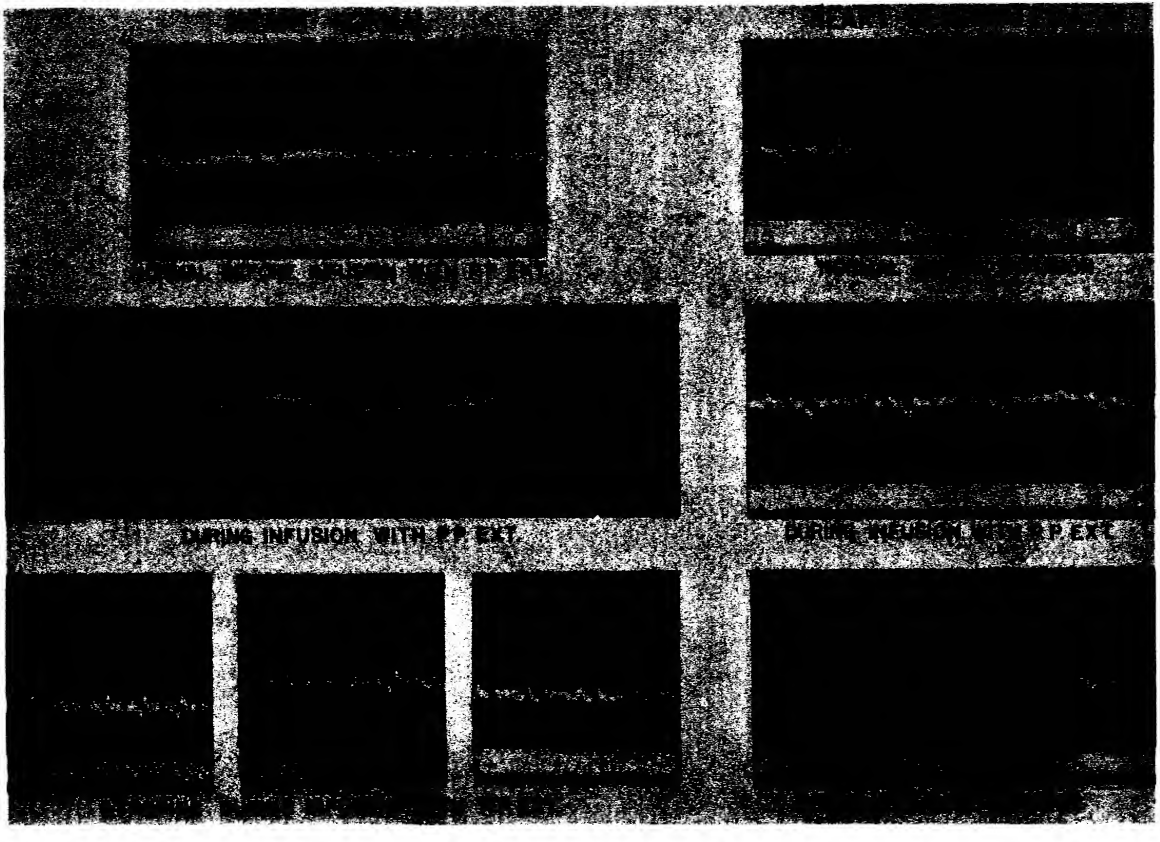


FIG. 6. *The effect of posterior pituitary infusion and the subsequent injection of atropine on the electrocardiogram (Lead II) of a dog before and after denervation of the heart. These records are from the animal that showed the maximal increase in basal heart rate after vagotomy.*

pressor effect. Anaesthetics, particularly chloretone, intensify the pressor action (3, 10). Coronary dilators such as ephedrine and adrenaline also increase the pressor effect of small doses and may nullify the depressor effect of large doses (8).

The effect of infusion of posterior pituitary extract on blood pressure was registered in seven normal unanaesthetized dogs. (For examples, see Table II, Fig. 4A). A pressor effect of 10 to 30 mm. of mercury was obtained within 30 sec. in six unanaesthetized dogs, and a fall of 5 mm. in one. The maximal cardio-inhibition followed the maximal pressure rise, but the heart was slowed even in the animal that showed the depressor effect. The pressure slowly fell with continued infusion, sometimes reaching the pre-infusion level. The bradycardia was maintained however, and the rate did not return to the resting level until 20 min. after cessation of infusion. These results indicate that the vagal effect does not depend on a depressor reflex.

B. Dogs with Thoracic Sympathectomy

Thoracic sympathectomy, with removal of stellate ganglia, was done in four dogs. This did not alter the resting-heart rate of 60 to 90 per minute. Two weeks after the final operation, posterior pituitary infusions were given. This produced an inhibition indistinguishable from that in the normal animal (Table I), with normal blood pressure elevation (Table II) and electrocardiographic effects, and with normal recovery time. Bacq and Dworkin (1) found that single injections of pitressin in the completely sympathectomized cat produced the same inhibition as in the normal cat, although the recovery time was prolonged.

Atropine injected during the bradycardia produced by infusion caused the typical tachycardia seen in the normal dog.

C. Vagotomized Dogs

Samaan (13) and others found that section of both cervical vagus nerves in the anaesthetized dog is followed by extreme cardiac acceleration. A similar increase in basal heart rate has been reported for the unanaesthetized cat (1, 9).

In this investigation bilateral vagotomy was done in three dogs whose response to posterior pituitary infusion, with and without atropine, had been established. Two of these had higher resting-heart rates than had most of the experimental animals. No change in heart rate occurred after section of one vagus. After double vagotomy one animal showed no change in basal heart rate (Fig. 1), the second showed a slight rise, and the third, a considerable acceleration (Table III). This third animal had respiratory irregularities probably due to inability to accommodate in the short interval (six days) between section of the two nerves.

TABLE III

THE EFFECT OF POSTERIOR PITUITARY EXTRACT ON HEART RATE BEFORE AND AFTER VAGOTOMY

Dog Sally			Dog Katy			Dog Sambo		
Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate	Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate	Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate
Before vagotomy			Before vagotomy			Before vagotomy		
102	48		110	60		90	40	
100	56		94	43		74	36	
84	56		124	56		84	44	
102	48		96	60		66	34	
88	56	42			51	74	37	39
After unilateral vagotomy			After unilateral vagotomy					
102	83		102	54				
96	68		102	56				
90	68	23			47			
After double vagotomy			After double vagotomy			After double vagotomy		
104	92		132	108		138	122	
100	86		124	104	27	148	128	18
90	76							
96	80							
98	80							
134	118	15						

The Effect of Posterior Pituitary Infusion on the Heart Rate

After double vagotomy the inhibition produced by posterior pituitary infusion was considerably less than that which occurred in the normal animal (Table III). Its onset was delayed and did not reach a maximum until 20 min. after the start of infusion (Fig. 1). It was then maintained but gradually ceased after withdrawal. Blood pressure changes during infusion were similar to those obtained in the normal animal (Table II, Fig. 4B). Infusion produced the following electrocardiographic changes (Fig. 5): lengthening of the P–R interval, inversion of T wave, increase in T voltage. There was no grouping of beats. The inversion of the P wave reported by Melville (7) was not seen.

The Effect of Atropine on Posterior Pituitary Inhibition

Atropine has been reported to cause cardiac acceleration after double vagotomy in the cat (9) and the dog (12).

We were not able to demonstrate this at any time in three doubly vagotomized dogs, either immediately after section or two weeks later. When atropine was given while the heart was inhibited by pituitary infusion, there was no acceleration (Fig. 1). Given before infusion, atropine did not inter-

fere with the inhibitory action of posterior pituitary extract. Atropine did not alter the electrocardiogram of the vagotomized dog under influence of posterior pituitary extract (Fig. 5).

It is significant that in the dog with a sympathetic cardiac denervation only, posterior pituitary infusion still exercises an inhibition on the heart, even in the presence of atropine.

D. Dog after Unilateral Vagotomy and Bilateral Thoracic Sympathectomy

Several experiments were done on one animal after recovery from single vagotomy and double thoracic sympathectomy.

Infusion with posterior pituitary extract produced a slowing of the heart equivalent to that produced when the same heart had its normal innervation.

Atropine caused the same acceleration as was produced before the partial denervation, and overcame the bradycardia produced by posterior pituitary infusion just as in the normal dog.

E. Animals with Complete Denervation of the Heart

Bacq and Dworkin (1) produced no change in heart rate with single injections of pitressin in the cat immediately after total denervation, but observed a considerable acceleration of the chronically denervated heart.

TABLE IV

THE EFFECT OF POSTERIOR PITUITARY EXTRACT ON HEART RATE BEFORE AND AFTER COMPLETE DENERVATION OF THE HEART

Dog Barney			Dog Colonel		
Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate	Resting heart rate per min. on different days	Minimal infusion rate per min.	Average fall in heart rate
Before denervation			Before denervation		
104 90 84 102 86	38 36 34 40 36	56	90 90 68 62	54 42 34 31	37
Both sympathetic chains removed, 1 vagus cut					
82 88 82	36 48 48	40			
Heart completely denervated			Heart completely denervated		
92 96 94	94 102 90	Nil	92 82	88 78	4

Experiments were done on two animals after operative recovery from total cardiac denervation. Infusion with posterior pituitary extract produced no characteristic slowing of the heart rate in five experiments in our dogs, 1 to 10 days after total denervation (Table IV, Fig. 2). It caused the characteristic rise in blood pressure (Fig. 4C). Denervation did not alter the electrocardiogram from the normal, and infusion now made no change in it (Fig. 6).

Atropine alone or in combination with pituitary infusion had no effect on the heart or electrocardiogram (Fig. 6).

Discussion and Conclusions

Our results indicate that the inhibitory action of physiological doses of pituitary extract in the unanaesthetized dog is *solely* through the cardiac nerves. This is contrary to the opinion of several investigators who conclude that posterior pituitary extract acts, not only on the vagus mechanism, but also by direct myocardial depression, or coronary arterial constriction, or both.

Our experiments indicate that both sympathetic and vagus fibres are involved, since after vagotomy the extract is still able to cause some inhibition, but only so long as the sympathetic innervation is retained. Cardioinhibitory fibres have been demonstrated in the sympathetic supply to the dog heart (5); these are not affected by atropine. In the normal animal posterior pituitary inhibition is probably mainly through the vagus, since interruption of the sympathetic supply does not diminish it.

It is unlikely that the nervous inhibition is due to a carotid sinus-depressor reflex, since it may be initiated with no rise or only a slight rise in blood pressure (Table I).

Our experiments do not demonstrate whether the stimulation of inhibitory fibres is central or reflex from the heart. If reflex, afferent as well as efferent fibres must exist in both sympathetic and parasympathetic pathways.

We do not deny that posterior pituitary extract can cause coronory arterial constriction, by direct action, but this does not seem to be a primary factor in causing inhibition, for total denervation prevents the inhibitory effect of the extract. If constriction were to activate sensory receptors in the walls of the arteries, this might produce reflex inhibition which would be abolished by denervation.

Since total denervation abolishes the inhibitory effects, it is unlikely that posterior pituitary extract acts by myocardial depression. Support to this view is provided by the observation that the typical electrocardiographic signs that accompany the inhibition of the normal heart are abolished when the extract is infused after total cardiac denervation.

Our results with the dog are in partial agreement with those of Bacq and Dworkin with the cat, in which complete denervation abolished the inhibitory effect of pitressin. They found, however, that pitressin produced acceleration of the chronically denervated heart. This was never observed in our dogs either immediately after total denervation, or for two weeks thereafter.

References

1. BACQ, Z. M. and DWORKIN, S. Am. J. Physiol. 95 : 605-613. 1930.
2. CANNON, W. B., NEWTON, H. F., BRIGHT, E. M., MENKIN, V., and MOORE, R. M. Am. J. Physiol. 89 : 84-107. 1929.
3. GRUBER, C. M. J. Pharmacol. 36 : 155-172. 1929.
4. GRUBER, C. M. and KOUNTZ, W. B. J. Pharmacol. 39 : 275-299. 1930.
5. HERMANN, H., JOURDAN, F., and FROMENT, R. Compt. rend. soc. biol. 128 : 673-676. 1938.
6. MELVILLE, K. I. J. Pharmacol. 47 : 355-363. 1933.
7. MELVILLE, K. I. J. Pharmacol. 64 : 86-110. 1938.
8. MELVILLE, K. I. and STEHLE, R. L. J. Pharmacol. 42 : 455-470. 1931.
9. MOORE, R. M. and CANNON, W. B. Am. J. Physiol. 94 : 201-208. 1930.
10. RAGINSKY, B. B., ROSS, J. B., and STEHLE, R. L. J. Pharmacol. 38 : 473-480. 1930.
11. RESNIK, W. H. and GEILING, E. M. K. J. Clin. Investigation, 1 : 217-238, 239-245. 1925.
12. ROGERS, F. T. Am. J. Physiol. 53 : 15-24. 1920.
13. SAMAAN, A. J. Physiol. 83 : 332-340. 1935.
14. WAKIM, K. G., HERRICK, J. F., BALDES, E. J., and MANN, F. C. J. Lab. Clin. Med. 27: 1013-1022. 1942.

THE PRODUCTION OF CANNED PRECOOKED CHICKEN[1]

By E. J. Reedman[2]

Abstract

Three classes of raw dressed chicken were processed by open and pressure precooking methods, and tested by a consumer taste panel. Canned chicken of good quality was obtained from even the lowest grade of raw dressed chicken used, but the higher grades of raw material produced packs of superior quality. However, such quality was dependent on efficient processing. The results suggest that the quality of Canadian canned chicken could be improved by the uniform adoption of pressure precooking methods by the industry. The use of pressure precooking would also assist in the production of a more uniform pack, because of lower moisture content of the cooked meat, smaller volume of broth and shorter cooking periods. Control measures are necessary to ensure uniformity of product, particularly in such attributes as the weight of meat packed, the concentration of broth used, and the strength of jelly in the final product. Suitable methods and apparatus are described.

Introduction

As a result of the increased war-time demand for eggs, Canadian production of canned precooked chicken (also known as canned sliced or canned boned chicken) has developed considerably in recent years. A survey of commercial products indicated that quality improvement was possible in many, while a lack of uniformity even within the same brand definitely pointed to a need for closer control in manufacture. In the present investigation, therefore, attention was directed towards improvement of the quality of Canadian canned chicken, and the production of a more uniform pack.

Factors Affecting Quality of Product

Grades of raw material and the cooking methods used were considered to be the most probable factors having appreciable effects upon the quality of canned precooked chicken.

Materials

The canning industry naturally prefers to use the least expensive (i.e., lowest grade) raw material available, provided that it produces a finished product of desirable quality. Since the top grades for chicken set by the Canadian Standards (1) are strongly influenced by such factors as the colour of the fat and the symmetry of the bones, this attitude of the canners appeared to merit consideration, particularly as both bones and fat are removed before the chicken is canned.

In the present investigation, three classes of raw dressed chicken were used: Class *A* was a composite of the three top grades, namely, Special

[1] *Manuscript received June 30, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 97 of the Canadian Committee on Food Preservation, and as N.R.C. No. 1143.

[2] *Biochemist, Food Investigations.*

Milk Fed, *A* Milk Fed, and *B* Milk Fed; Classes *B* and *C* were equivalent to standard grades *B* and *C;* however, raw material that had been graded down because of appearance factors was regraded for canning purposes.

Methods

The relative merits of open and pressure precooking also appeared to warrant investigation. Open precooking is the most widely used commercial method. Since the maximum temperature that can be reached is boiling point, prolonged cooking periods are required (60 to 90 min.). It is necessary to cover the meat in the kettle with water, which produces a large volume of broth, although this can be reduced by cooking several successive lots of meat in the same broth, or by concentrating. However, in pressure precooking, only sufficient water to generate steam need be added; this amounts to about 1 qt. per 25 lb. of meat. Also there is no loss from the kettle and a minimum exposure to oxidation. Since temperatures of 121° C. (250° F.) or even higher may be used, rapid cooking is obtained (15 to 30 min.).

Procedure and Results

Each of the three classes of raw material was precooked by both the open and pressure methods described, packed at the same weight of meat and strength of jelly in 7-oz. cans, and canned by the same process. This series of six packs was produced in a commercial establishment. A taste panel consisting of 30 members was used to assess the quality of the finished product. In this test comparisons between all packs were made by tasting two samples at a time.

Table I shows that, when the results are averaged over the entire experiment, neither the class of raw material nor the method of precooking had significant effects on eating quality. However, when the classes are considered in detail, as averaged over both precooking methods, it is evident that Class *C* chicken was significantly inferior to Classes *A* and *B*, and that no difference could be distinguished between *A* and *B* classes. Detailed analysis of the differential quantities shows, however, that when pressure precooking was used, Class *A* chicken was distinguished as being significantly superior to the other classes.

These results indicate that three classes of chicken can be distinguished when prepared by the pressure precooking methods used here, but only two classes can be distinguished when open precooking is used. The higher quality of first grade raw material can be retained only by the use of pressure precooking. It has also been shown that pressure precooking results in a higher retention of vitamin C (3).

Factors Affecting Uniformity of Product

Since uniformity is essential in maintaining brand consistency, it was considered desirable to apply control measures wherever feasible. Factors that appeared amenable to control were: the proportion of meat to jelly, the concentration of broth used, and the strength of jelly in the final pack.

TABLE I

RESULTS OF QUALITY TESTS ON CANNED CHICKEN

Ratings assigned by a flavour test panel of 30 members

Cooking method	Class of chicken			Total	$A + B$
	A	B	C		
Open	−10	8	−17	−19	− 2
Pressure	36	5	−22	19	41
Total	26	13	−39	0	39

Analysis of variance

Source of variance	Degrees of freedom	Mean square
Treatments	5	5.02
Class	2	6.57
C vs. $A + B$	1	12.68*
A vs. B	1	0.47
Cook	1	2.67
Class × cook	2	4.89
C vs. $(A + B)$ × cook	1	2.60
A vs. B × cook	1	6.67
A vs. $(B + C)$ × cook	1	9.26*
B vs. C × cook	1	0.01
Error (b)†	145	2.33*
Error (a)	300	1.75

† *Error (b) used to test the significance between treatments and between its various components.*
* *Exceeds 5% level of statistical significance.*

Ratio of Meat to Jelly

The quality of a canned food is affected by the quantity of solids or concentrate in proportion to water or other filling material present. For canned chicken, 50% by weight of meat appears to be a reasonable lower limit. The use of pressure precooking enables all the broth produced to be packed. Pressure and open precooked meat contain 60 to 65% and 70 to 75% moisture, respectively, and since the retorting process has been found to reduce the higher moisture content to approximately 60 to 65%, adjustments should be made when packing open precooked meat.

A separation device suitable for checking weights of meat and jelly should be capable of being heated under pressure to liquefy the jelly, and vapour-tight to prevent the loss of volatile materials. Such an apparatus is shown in Fig. 1. The apparatus consists essentially of upper and lower receptacles joined with an air-tight gasket, and upper and lower plates that can be used to draw the receptacles together by means of bolts and wing nuts. The bottom of the upper receptacle consists of 60-mesh stainless steel screening. The previously weighed receptacles are assembled, the contents of a can

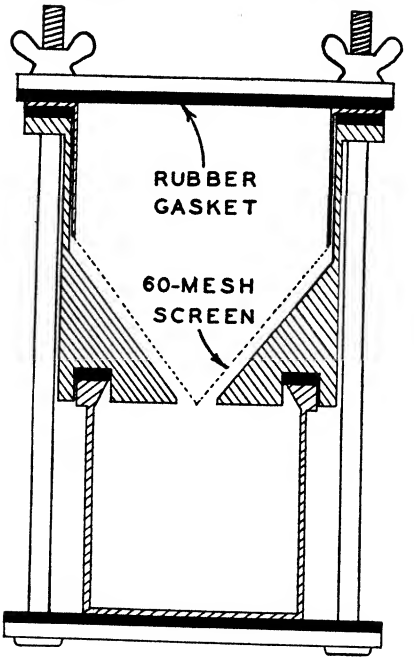


FIG. 1. *Apparatus used for separating meat and jelly in canned chicken.*

weighed into the upper receptacle, and the entire apparatus heated under pressure to liquefy the jelly. After heating, the separation apparatus is cooled, the receptacles are weighed, and the weights of meat and jelly determined.

It was found that a 30-min. heating period at 121° C. (250° F.) with a subsequent cooling of 30 min. in cold water, gave a complete separation of a 7-oz. net weight pack. Moreover, this procedure gave a meat of approximately the same moisture content as that after retorting. Table II gives typical data on the proportions of meat and jelly obtained in laboratory and commercial tests.

Quality and Strength of Broth

In canned chicken the broth obtained from the meat during precooking is an important portion of the final product: not only does the broth contribute to the food value of the product, but it also contributes to flavour. The quality of broth depends upon the grade of raw material and the method of preparation. From the quality tests recorded in Table I, it appears that pressure precooked meat yields a broth of better flavour than that prepared by open precooking. Heating treatments used for concentrating or for dissolving gelling agents are deleterious to broth quality.

TABLE II

SEPARATIONS OF MEAT AND JELLY IN FINISHED CANS OF PRECOOKED CHICKEN

Class of sample	Net weight, oz.	Weight of meat, oz.	Weight of jelly, oz.	Moisture in meat after separation, %
Laboratory samples	7.0	3.5	3.5	63
	6.9	3.5	3.4	63
	7.0	3.6	3.4	64
	7.0	4.0	3.0	62
	7.0	4.1	2.9	63
Commercial samples	7.2	3.2	4.0	63
	7.3	3.5	3.8	64
	7.2	3.0	4.2	61
	7.1	2.9	4.2	62
	7.0	2.8	4.2	63
	6.9	1.9	5.0	62

It was considered desirable to measure broth concentration in terms of solids present. Two methods for measuring the total solids present were considered feasible, namely: the evaporation of a measured volume of broth to dryness and determination of the amount of solids remaining, or preferably, the measurement of the specific gravity of the broth and its correlation with actual solids found by the first procedure.

It has been commercial practice to separate the fat from the broth after precooking, since if fat is packed with the broth it will rise to the top in the can and give an undesirable flavour and appearance to the finished product. Measurements of broth concentration are therefore made after separation of the fat and before addition of salt, gelling agent, or other substance to the broth. Sodium chloride was determined by the Mohr method after ashing the samples in a muffle furnace. Solids corresponding to specific gravities from 0.990 to 1.040 at 50° C. (122° F.) were measured, enabling determination of the correlation of specific gravity and solids in any broth likely to be encountered in commercial practice.

Table III shows specific gravities obtained in samples of broth prepared by both open and pressure precooking methods. From a large number of laboratory and factory measurements it was considered that the specific gravity should not fall below 1.000 or 1.010 at 50° C. (122° F.) for open and pressure precooked broths, respectively, if a desirable strength of broth was to be packed.

Quality and Strength of Jelly

Meat broth contains natural gelatine which contributes to the strength of the final jelly, but such natural gelatine has a tendency to hydrolyse or break down in the commercial sterilization of the product after packing. Consequently it is now general practice to add further amounts of a gelling agent such as agar or gelatine to obtain a desirable strength of jelly. Agar should

TABLE III

SPECIFIC GRAVITY AND SOLIDS CONTENT OF BROTHS FROM SAMPLES OF PRECOOKED CHICKEN[1]

Method of precooking	Specific gravity at 50° C. (122°F.)	Weight of solids, oz. per gal.	Method of precooking	Specific gravity at 50° C. (122°F.)	Weight of solids, oz. per gal.
Pressure	1.021	15.9	Open	1.002	5.5
	1.0185	14.5		1.002	5.5
	1.0182	14.3		1.001	4.8
	1.0175	13.1		1.000	4.0
	1.0125	11.2		1.000	4.0
	1.011	10.0		1.000	4.0

[1] *These varying strengths of broth were obtained by different times of precooking. In commercial practice it is possible to obtain fairly uniform solids between batches. Open precooked broths had only one lot of meat cooked in them.*

not be added in a concentration of above 3% by weight in the final broth; two to two and one-half per cent is usually adequate (2). If gelatine is used the grade must be carefully chosen, to prevent excessive hydrolysis on retorting. Typical data on the solids content of the jelly are given in Table IV. It is of importance to note that during retorting additional solids come out of the meat into the broth.

TABLE IV

SOLIDS IN THE BROTH AND JELLY OF CANNED CHICKEN

Broth after precooking		Broth after addition of agar and salt		Final jelly after retorting	
Specific gravity at 50° C. (122°F.)	Solids content, oz. per gal.	Specific gravity at 50° C. (122°F.)	Solids content, oz. per gal.	Specific gravity at 50° C. (122°F.)	Solids content, oz. per gal.
1.009	8.6	1.013	12.1	1.025	17.5
1.010	9.5	1.020	15.6	1.033	21.0
1.010	9.5	1.020	15.6	1.030	20.0
1.011	10.0	1.0205	15.8	1.033	21.0
1.011	10.0	1.021	15.9	1.030	20.0

The final jelly strength may be tested as follows: the jelly and meat are separated in the apparatus described and the jelly allowed to solidify in the lower receptacle. (Separation weakens the jelly somewhat, but since this is a constant factor it can be disregarded.) The jelly is conditioned for three hours at 7° C. (45° F.). The apparatus used for determining jelly strength (Fig. 2) consists essentially of an oil-damped trip scale with the pointer extended to a length of one foot. The receptacle containing the jelly is placed on one pan and the scale balanced at zero by adjustment of the beam weights. A cone-shaped plunger mounted above the jelly is so adjusted that its tip just touches the surface of the jelly. Mercury is run into a container

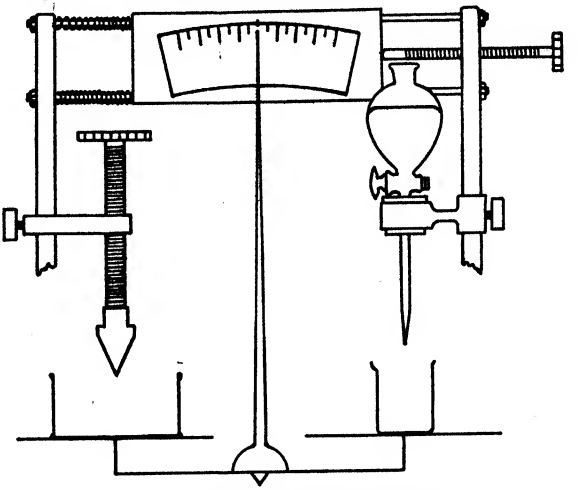


FIG. 2. *Device for testing jelly strength.*

on the other pan to force the jelly against the plunger a standard distance, as shown by the deflection of the pointer. Thus jelly strength is measured by the resistance of the jelly to the penetration of the plunger, and is expressed as grams of mercury. Table V shows typical results obtained with this device.

TABLE V

MEASUREMENTS OF JELLY STRENGTH

Class of sample	Description	Observations on jelly	Jelly strength, gm. mercury	
			Range	Average
Laboratory samples	1% agar in broth	Did not gel	—	—
	2% agar in broth	Fairly firm jelly	13.0—15.7	15.1
	2% agar in broth	Fairly firm jelly	15.7	15.7
	2.5% agar in broth	Firm jelly	15.6—35.5	26.6
Commercial samples	—	Very weak jelly	5.3—10.1	8.0
	—	Very weak jelly	6.5— 6.9	6.6
	—	Very weak jelly	6.2— 7.2	6.7
	—	Weak jelly	9.7—12.5	10.7
	—	Fairly firm jelly	14.4—14.9	14.5
	—	Firm jelly	25.5—32.3	28.2

Acknowledgments

Grateful acknowledgment is made to the staff of the Poultry Marketing and Inspection Services, Dominion Department of Agriculture, whose able assistance facilitated this study, to Mr. M. W. Thistle, National Research

Laboratories, who aided in the preparation of the manuscript, and to Mr. Leonard Buckby, Laboratory Assistant, who prepared the figures.

References

1. CAN. DEPT. AGR. Regulations under the provisions of the Live Stock and Live Stock Products Act, Chapter 47, of the Statutes of Canada, 1939, respecting the Grading and Marking of Dressed Poultry. (As published in the Canada Gazette, April 3, 1943.)
2. REEDMAN, E. J. Unpublished data.
3. REEDMAN, E. J., BUCKBY, L., and LEGAULT, R. T. Can. J. Research, D, 20 : 230-234. 1942.

DRIED WHOLE EGG POWDER

VII. EFFECT OF TEMPERATURE AND MOISTURE ON THE BACTERIAL CONTENT OF LIQUID AND DRIED EGG[1]

By N. E. Gibbons[2] and C. O. Fulton[2]

Abstract

The bacterial content of liquid egg increased rapidly after about six hours at 20° C., 12 hr. at 15.6° C., 25 hr. at 11.1° C., and two to three days at 7.2° C. At 3.3° C. there was little change for five to six days, followed by a very gradual increase.

The bacterial content of dried egg powder was influenced by the number of bacteria in the melange, the drying temperature, the rate of cooling, the storage temperature, and the moisture content. Low drying temperature and rapid cooling of the powder favoured survival. On storage the bacterial mortality increased with increasing time and temperature. At temperatures above 30° C. the death rate seemed to be proportional to changes in temperature. At 7.2° C. and lower the majority of organisms survived eight months' storage. Up to 8.6%, moisture content had little effect on bacterial survival. At moisture levels above 5% there was an increase in the number of moulds, particularly at 23.9° and 32.2° C.

Introduction

Although there is as yet no direct evidence that any correlation exists between bacterial content and the quality of dried egg powder, the number of bacteria present in the powder gives some indication of the sanitary conditions prevailing in the breaking and drying plants. Information on the effect of plant practices on survival or increase of organisms during the storage and drying of melange and the cooling and storage of powder, presented here, was collected during actual plant operation and in the laboratory. Most of the data is concerned with dried egg. However, some experiments on the effect of temperature on the bacterial content of the egg liquid are also included, since the load in the liquid determines to a large extent the bacterial content of the powder.

Methods

The number of organisms in liquid egg was estimated by shaking 10 ml. of the melange with 90 ml. of water and making the appropriate dilutions.

The method of making counts on powder has been described (3). Plates were poured using proteose-peptone, tryptone agar, and counts were made after incubating for three days at 37° C.

Comparisons were made between the above agar and the tryptone glucose agar without the addition of milk that is usually recommended for the examination of egg products (1). Duplicate samples from 20 carlots were plated on

[1] *Manuscript received July 19, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 99 of the Canadian Committee on Food Preservation and as N.R.C. No. 1154.

[2] *Bacteriologist, Food Investigations.*

both media and incubated for three days at 32° C. and 37° C. An analysis of variance indicated no significant difference between the counts obtained on the two media. However, there was a significant difference in the number of organisms at the two incubation temperatures. This was due to the fact that on about half the samples the count at 32° C. was significantly higher than at 37° C. It would appear therefore that the flora of egg powders may differ and that the lower incubation temperature favours the development of certain of these organisms.

Results

Liquid Egg

In an initial experiment to determine the temperature at which liquid egg may be held with safety, melange prepared from commercial eggs was stored for periods up to 21 days at 3.3°, 7.2°, 20°, 23.9°, and 37° C. (38°, 45°, 68°, 75°, and 98° F.).

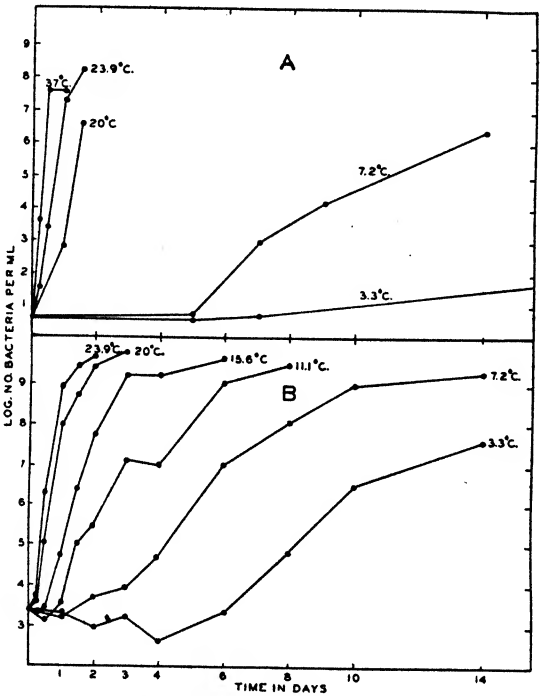


Fig. 1. *Increase in number of bacteria in liquid egg held at various temperatures.*

Although the original count was less than 10 organisms per ml., the count rose rapidly at room temperature and above (Fig. 1A). However, at 7.2° C. there was no appreciable increase for five days and the pH changed little from that of the original (7.5). The melange stored at 37° C. coagulated in 24 hr. (final pH 5.4), in 36 hr. at 23.9° C. (pH 5.7) and in 48 hr. at 20° C. (pH 5.7).

Since it was considered possible that growth might be rapid at temperatures slightly above 7.2° C., a second experiment was carried out using melange with a somewhat higher initial count. This material was incubated at 3.3°, 7.2°, 11.1°, 15.6°, 20°, and 23.9° C. for periods up to 14 days. It was again observed that the rate of bacterial development increased as the temperature increased (Fig. 1B). At 7.2° C. a fairly rapid increase took place after a lag of about three days and after approximately six days at 3.3° C.

It would appear that a different flora was present in this lot of melange since even at 23.9° C. with a bacterial content of some thousand million per ml. at 24 hr., the pH dropped to only 6.7 and then increased to 7.1. This drop and rise in pH was noted at all temperatures above 11.1° C. The more rapid growth at lower temperatures than in the previous experiment would also indicate a different flora.

From the above results it would appear that under normal sanitary and storage conditions egg melange can be held for 24 hr. and possibly 48 hr. at 7.2° C. without the bacteria increasing appreciably. At a temperature of 11.1° C. melange cannot be held safely for even 24 hr. and at 20° C. for 12 hr. unless the initial count is extremely low.

Dried Egg Powder

Effect of Temperature of Drying

The temperature at which the liquid egg is dried is an important factor in determining the quality of the powder produced (7). Within limits, the lower the drying temperature the better the quality of the powder, but, as might be expected, more bacteria survive. This is shown in Table I, where

TABLE I

Effect of drying temperature on the bacterial content of egg powder

Plant	Outlet temperature, ° C.	No. of organisms per gram	Outlet temperature, ° C.	No. of organisms per gram
A	65	8900	51.7	26,500
B	68.3	26,000	54.4	130,000
C	57.2	112,000	52.2	338,000

the bacterial content of powder dried at two different temperatures in three plants is recorded. A decrease of from 4° to 15° C. in the temperature of the outlet air is accompanied by a three- to fivefold increase in the number of bacteria in the resulting powder.

Effect of Cooling

Rapid cooling of the powder after leaving the drier is important if high quality is to be maintained (4). Slow cooling, on the other hand, will usually result in powders with lower bacterial counts. For instance, it has been observed that samples removed from the top and bottom layers of powder,

collected in the bottom of a box type drier having an outlet temperature of 51.8° C., had different counts. The bottom layer, which had been exposed to this temperature for about an hour, had a count of 6700 per gram as compared with 32,000 in the top layer which had been exposed for a shorter time.

In an initial experiment to study the effect of cooling rates, changes in the bacterial content of the powder in two barrels held at 5.6° C. were followed. The barrels were filled with powder at 40.6° C., headed immediately, and placed at 5.6° C. for four days. The powder near the outside of the barrel was below 21° C. in six hours, 9.4° C. in 24 hr., and 5.8° C. in 48 hr. In the centre the temperature remained at 40.6° C. for six hours and dropped to 36° C. after 24 hr. After two and a half days the centre was just below 21° C. and after four days, 12.2° C. The barrels were stored a further seven days at 26.7° C. before sampling. The bacterial content of one barrel decreased from an initial count of 17,000,000 per gm. to 62,000 per gm. in the centre and 190,000 at the outside. In the second barrel an initial count

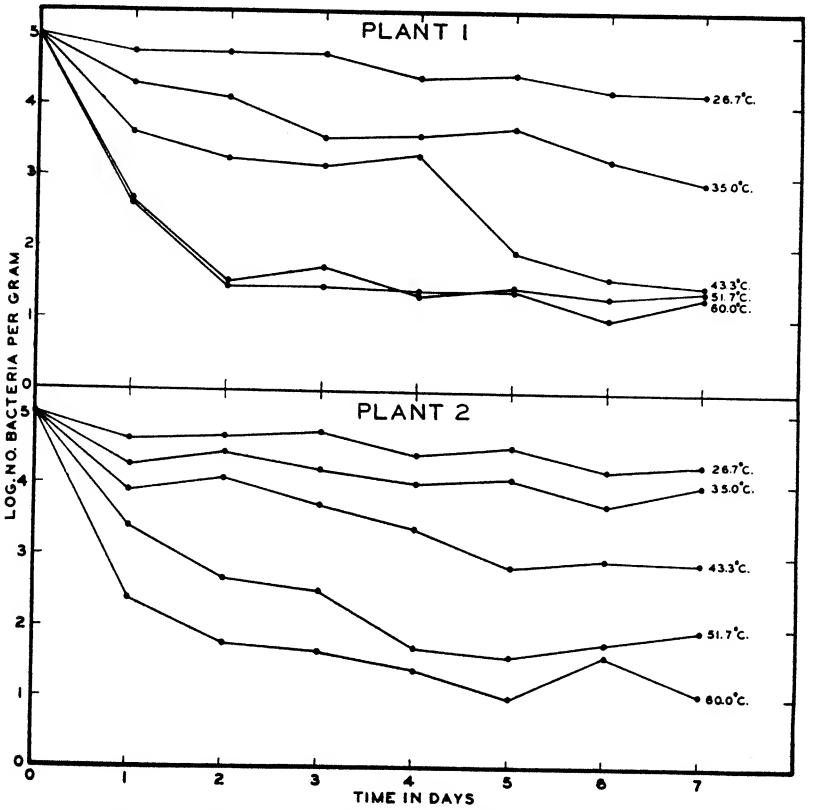


FIG. 2. *Changes in the bacterial content of egg powder held at 26.7°, 35.0°, 43.3°, 51.7°, and 60° C. for seven days.*

of 10,000,000 per gm. decreased to 30,000 in the centre and 190,000 at the outside. This differential effect indicated that cooling is an important factor in determining the bacterial content of dried egg powder.

Since cooling rates are difficult to control in practice, pertinent information was obtained by holding powders from two plants at 26.7°, 35°, 43.3°, 51.7°, and 60° C. for 3, 6, 12 hr. and one, two, three, four, five, six, and seven days (4).

The effect on the bacterial content is shown in Fig. 2. At 60° C. over 99% of the organisms were destroyed during the first day. At 51.8° C. the greatest reduction also took place during the first day. However, in the same time the quality of the powder deteriorated considerably (4). At 43.3° and 35° C. there was a gradual but definite decrease with time but at 26.7° C. there was only a very slight decrease over the seven days. It is therefore evident that if the powder were cooled rapidly enough to preserve quality the majority of the organisms surviving the drying process would also be preserved.

Effect of Storage Temperature

Powder from one plant was stored for 36 wk. at 0.56°, 7.2°, 20°, and 37° C. and at 55° C. for 10 wk. The results are presented in Fig. 3. At 0° C. there was little change in the bacterial content. At temperatures of 7.2° C. and higher, the rate of bacterial decrease was proportional to the increase in

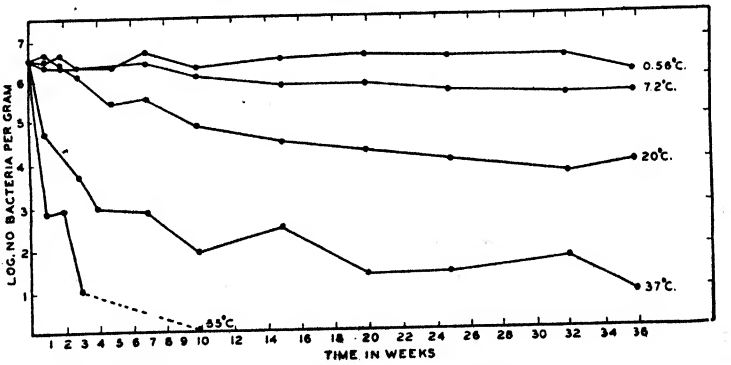


FIG. 3. *Changes in the bacterial content of egg powder held at 0.56°, 7.2°, 20°, 37°, and 55° C. for periods up to 36 wk.*

temperature. At 37° C. there was a decided decrease during the first four weeks, with a more gradual change thereafter. At 55° C. the powder was practically sterile after four weeks since less than five organisms per gm. were present. At the 10th week it was found, by the dilution method, that there was about one organism per gm. These were spores.

In a second storage experiment powders from three producers were stored at 7.2°, 15.6°, and 23.9° C. for eight months and at 32.2° C. for three and a half months. The results (Fig. 4) in general are in agreement with those of

the preceding experiment. The greater reduction in bacterial content at 32.2°, as compared with 23.9° C. and lower, is of interest since a similar observation was noted for changes in quality (6).

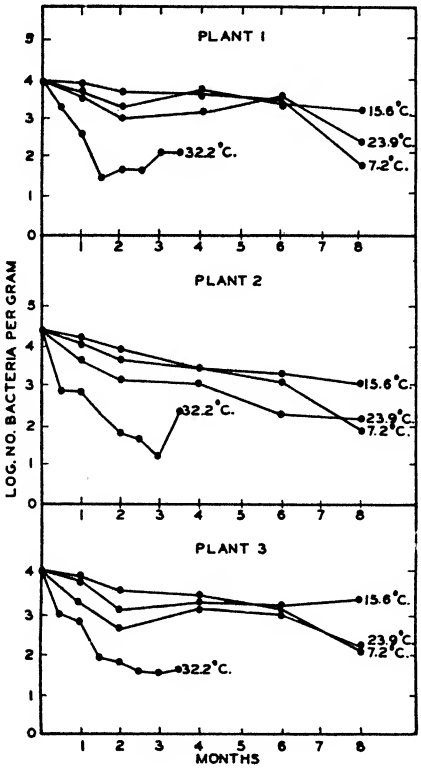


FIG. 4. *Effect of storage at 7.2°, 15.6°, 23.9°, and 32.2° C. for periods up to eight months on the bacterial content of egg powder from three plants.*

The moisture content of the powder stored in convolutely wound, paper-bodied containers held at 7.2° C. began to increase about the fourth month (6). By the sixth month the moisture level had increased about 2% and by the eighth month about 9%. The decrease in the bacterial content of this powder was apparently linked with the change in moisture content.

Effect of Moisture Content

Powder from a commercial drier was adjusted to moisture levels of 3.07, 3.7, 5.3, and 8.6% (5) and stored at 7.2°, 15.6°, 23.9°, and 32.2° C. At 32.2° C. examinations were made semimonthly for two months and at the lower temperatures after one, two, four, and six months.

The results are shown in Fig. 5. Although an analysis of variance of the data indicated that there was a significant difference in the bacterial content of the powders with different moisture contents it is evident that much of this is due to differences in the original bacterial population of the powders. The

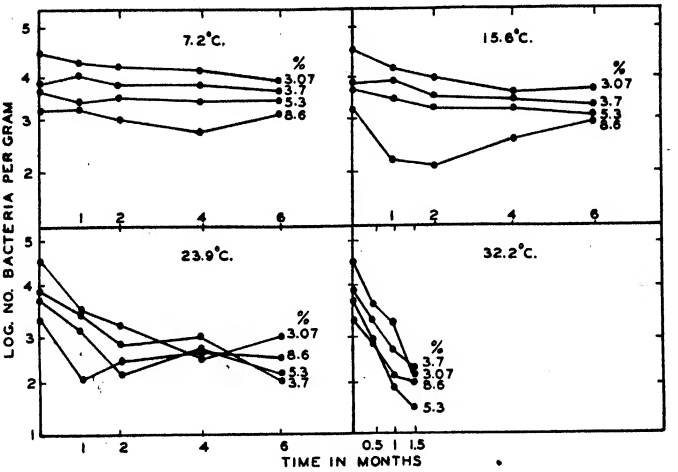


FIG. 5. *Effect of 3.07, 3.7, 5.3, and 8.6% moisture content on the bacterial content of egg powder stored at four temperatures.*

powder adjusted to 3.07% moisture was handled considerably while bringing it to a lower moisture level which may have been responsible for the high count. It is not known why raising the moisture content to 8% resulted in a lowering of the bacterial content although, as mentioned in the preceding section, the bacterial content of powders held at 7.2° C. began to decrease when the moisture content began to increase. Changing moisture levels appears to have more effect on the bacterial content than constant levels. It would seem that at the levels studied, once the moisture content has been adjusted, temperature is the more important factor in determining the change in bacterial content.

In contrast to the behaviour of bacteria there was an increase in the number of moulds at 5% and particularly at 8% moisture. This was more noticeable at 23.9° and 32.2° C. but in some instances numerous moulds were observed on the plates of powders stored at 7.2° C.

The same behaviour was noted in a study of containers. In one container in which the moisture content of the powder increased from 3.6 to 7.5% the number of moulds increased and the number of bacteria decreased. In another instance where the moisture content increased from 3.6 to 16% in one month the powder was a mass of mould, and bacteria could not be detected on the plates. The storage temperature was 23.9° C. in both cases.

These data are not altogether in agreement with published observations that in 60 days at 30° C. the decrease in numbers of bacteria was least at about 5% moisture content and greater at the lower levels and at higher levels up to 10% (2). In the present studies at 32.2° C. it has been found that there is a steady decrease in numbers at all constant moisture levels used. The irregular results obtained at the higher temperatures with the powder containing 8.6% moisture are probably fortuitous.

It has been noted, however, that when the moisture content changed during storage, as in the work of Stuart *et al.*, decreases in the numbers of bacteria were found. Such differences, however, occur in the types of organisms present in different lots of powder that it is difficult to generalize from a single experiment. Stuart *et al.* (2) did not find an increase in mould count at moisture levels below 10%. In the present study an increase in the number of moulds, usually accompanied by a decrease in the number of bacteria, was noted at all moisture levels at 32° C., although separate mould counts were not made.

Acknowledgment

The assistance of Mr. R. L. Moore, Laboratory Assistant, is gratefully acknowledged.

References

1. American Public Health Association. Standard methods for the examination of dairy products. 8th ed. New York. 1941.
2. Stuart, L. S., Hall, H. H., and Dicks, E. E. U.S. Egg Poultry Mag. 48 : 629-633, 658. 1942.
3. Thistle, M. W., Pearce, J. A., and Gibbons, N. E. Can. J. Research, D, 21 : 1-7. 1943.
4. White, W. H. and Thistle, M. W. Can. J. Research, D, 21 : 194-202. 1943.
5. White, W. H. and Thistle, M. W. Can. J. Research, D, 21 : 211-222. 1943.
6. White, W. H., Thistle, M. W., and Reid, M. Can. J. Research, D, 21. In press. 1943.
7. Woodcock, A. H. and Reid, M. In preparation.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. NOVEMBER, 1943 NUMBER 11

DRIED WHOLE EGG POWDER

VIII. AN IMPROVED FLUORESCENCE METHOD AND SOME FACTORS AFFECTING THE MEASUREMENT[1]

BY J. A. PEARCE[2], M. W. THISTLE[2], AND MARGARET REID[2]

Abstract

An improved method avoided prolonged filtration periods and effected a material saving in chemicals, but the results do not differ from those obtained by the original method. The improved method is as follows: 2.5 gm. of egg powder is defatted with three 25 ml. portions of chloroform; 1 gm. of the defatted powder is shaken for 30 min. with 100 ml. of 10% sodium chloride, filtered, and the fluorescence of 15 ml. of the filtrate determined in a photofluorometer.

Increase in temperature raised the values obtained by 0.65 photofluorometer units per Centigrade degree. Although decrease in the pH of the protein extracts was associated with increase in fluorescence values, variations in the pH of the protein solvent from 4.6 to 8.9 caused no significant change in fluorescence values.

Introduction

A fluorescence method as applied to dried whole egg powders was observed to be related to flavour quality (2, 3) and has been applied satisfactorily in the investigation of factors affecting quality (4, 5). However, the method was somewhat laborious for routine control work, mainly because of an excessively long filtering period. This paper describes an improved method designed to overcome these difficulties; and to give results comparable to those obtained by the original method. In addition, observations were made on the effect of temperature of extraction and pH of the protein solvent on the fluorescence values obtained.

Initial Experiments

Extraction of Fat

Some 13 fat solvents were tested in earlier work (1, 2). Of these, ethyl ether (1) appeared to warrant further study, as it gave equivalent readings and was considered somewhat less toxic than chloroform. Six samples, graded from 4 to 9 on a palatability scale (2, 3) were tested by the original method (2), except that the fat solvents and the number of fat extractions were varied.

[1] *Manuscript received June 30, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 100 of the Canadian Committee on Food Preservation and as N.R.C. No. 1155.

[2] *Biochemist, Food Investigations.*

Table I shows that, on the average, fluorescence values after two extractions did not differ significantly from those after three extractions when chloroform was used, and that the values obtained by three extractions with ethyl ether

TABLE I

EFFECT OF FAT SOLVENT AND NUMBER OF EXTRACTIONS ON FLUORESCENCE VALUES OF SIX SELECTED EGG POWDERS

Fat solvent	Mean fluorescence value
Chloroform	
Two extractions	39.85
Three extractions	38.65
Ethyl ether	
Two extractions	41.59
Three extractions	39.28
Necessary difference, 5% level of statistical significance = 1.48	

did not differ significantly from the values obtained with chloroform. However, ethyl ether was found to be much less convenient to use experimentally, and was given no further consideration.

Extraction of Protein

The original method (2) was based on an assumed total extraction of the fluorescent materials from the defatted powder. The first attempt at an improved procedure tested the equilibrium principle of extraction on six samples, as follows: 2.5 gm. of defatted powder was shaken with 100 ml. of 10% potassium chloride solution for periods of 5, 10, 15, 30, 60, and 120 min., made up to 250 ml. with 10% potassium chloride solution, mixed, filtered, and the fluorescence of the filtrate determined in the Coleman photofluorometer. Sufficient filtrate for reading purposes was obtained in 10 to 15 min. Table II shows that this method gave results significantly higher than those

TABLE II

EFFECT OF EXTRACTION TIME ON FLUORESCENCE VALUES OF SIX SELECTED EGG POWDERS

Method	Mean fluorescence values	
	Modification No. 1[1]	Modification No. 2[1]
Original (complete extraction)	41.62	40.82
Modified (equilibrium extraction)		
5 min.	43.52	—
10 min.	45.10	—
15 min.	44.63	—
30 min.	46.35	38.78
45 min.	—	40.93
60 min.	47.54	45.56
120 min.	46.66	—
Necessary difference, 5% level of statistical significance	1.75	2.29

[1] *Modification described in text.*

obtained by the original method. However, the data were of value in indicating maximum extraction of fluorescent materials in about 30 to 60 min. The greatly reduced filtering period suggested that a somewhat similar procedure might be satisfactory.

In a second attempt to utilize the equilibrium method of extraction, six samples were treated as follows: 1 gm. of defatted powder was transferred to a 250 ml. Florence flask, 100 ml. of 10% potassium chloride solution added, the mixture shaken for periods of 30, 45, and 60 min., filtered, and the fluorescence of the filtrate determined. Table II indicates that when the samples had been shaken for periods of 30 and 45 min., the results did not differ significantly from those obtained by the original method. The results from powders shaken for 60 min., in addition to differing significantly from the control method, did not agree closely between duplicate measurements. It would therefore appear that the equilibrium method of extraction for 30 to 45 min. can give satisfactory results.

Although 10% potassium chloride is commonly used as a protein solvent, previous work (1) had indicated 10% sodium chloride as a possible substitute. Since sodium chloride is cheaper and more easily obtainable than potassium chloride, both solvents were tested on six samples of powder, using the second modification (above) with 45 min. shaking. Table III shows no significant

TABLE III

EFFECT OF PROTEIN SOLVENTS (10% SOLUTIONS) AND METHOD OF EXTRACTION ON FLUORESCENCE VALUES OF SIX SELECTED EGG POWDERS

Method	Mean fluorescence value
Original	
Potassium chloride	40.82
Sodium chloride	44.14
Modification No. 2[1]	
Potassium chloride	40.94
Sodium chloride	41.25
Necessary difference, 5% level of statistical significance = 1.73	

[1] *Modification described in text.*

difference between the fluorescence values when sodium and potassium chloride were used in the modified procedure. No difference was evident between the new method, using either solvent, and the original method using potassium chloride, the results for the second modification in Table II thus being supported.

The effect of rate of shaking was evaluated on four egg powders of varying quality as follows: the defatted powders were extracted with sodium chloride solution by shaking at 100, 190, 290, and 390 impulses per minute (complete cycles) for 10, 20, 30, 40, 50, and 60 min. The effect of shaking time was

similar to that recorded in Table II. Mean fluorescence values were 40.4, 41.2, 42.7, and 43.5 for the shaking speeds noted. Although fluorescence appears to increase with increasing rates of shaking, these differences were not significant in comparison with the replicate error. Accordingly, 290 impulses per minute was chosen as a convenient rate.

Main Experiment

The foregoing preliminary experiments indicated that the number of fat extractions, the substitution of sodium for potassium chloride as protein solvent, and the length of the shaking period all merited further investigation. Consequently a more rigorous test was conducted on 16 powders consisting of two samples, each representing a carload lot of powder, prepared for export by each of eight Canadian egg-drying plants. All possible combinations of these factors were tested as follows: two and three defattings with chloroform, 10% potassium and sodium chloride solutions, the effect of 30 and 45 min. shaking by the equilibrium extraction method, and comparison of the equilibrium method with the original method.

Upon inspection of the results (Table IV) it was evident that the product from the four plants with the highest production rates was more variable than that from the remaining four plants. Therefore, in order to assess the significance of the various treatment differences, the plants were divided into two groups for purposes of statistical analysis. The only modified procedure

TABLE IV

EFFECT OF VARYING THE NUMBER OF DEFATTINGS, THE PROTEIN SOLVENT, AND THE METHOD OF PROTEIN EXTRACTION ON THE FLUORESCENCE VALUES OF 16 EGG POWDERS FROM EIGHT CANADIAN DRIED EGG PRODUCERS

Protein solvent	Procedure	Number of defattings with chloroform	Mean fluorescence values for groups of four plants	
			Group I	Group II
10% sodium chloride	Original	2	19.61	18.79
		3	17.20	16.80
	Modified, 45 min. shaking	2	19.41	19.06
		3	18.02	17.40
	Modified, 30 min. shaking	2	24.48	23.24
		3	16.94	17.26
10% potassium chloride	Original	2	17.59	17.28
		3	16.72	16.34
	Modified, 45 min. shaking	2	18.85	19.16
		3	18.16	18.04
	Modified, 30 min. shaking	2	20.79	20.01
		3	18.62	17.71
Necessary difference, 5% level of statistical significance			1.07	1.43

common to both groups that did not differ significantly from the original method involved three defattings with chloroform and protein dispersion by 30 min. shaking in 10% sodium chloride solution.

Modified Procedure

As a result of the foregoing work, an improved procedure is suggested for measuring the fluorescence of dried whole egg powder. According to this procedure, 2.5 gm. of egg powder is defatted at room temperature with three 25 ml. portions of chloroform (corks or stoppers are unsuitable), and filtered each time through a No. 1 Whatman filter paper. A fresh filter paper is used for the third extraction. After the final extraction, the defatted powder is allowed to dry at room temperature (about an hour), 1 gm. of defatted powder is transferred to a 250 ml. flask, and 100 ml. of 10% sodium chloride added. The mixture is shaken for 30 min. at approximately 300 impulses per minute, and filtered through No. 1 Whatman filter paper. The fluorescence of a 15 ml. portion of the filtrate, usually obtained in 10 to 15 min., is determined in the Coleman photofluorometer.

The Effect of Temperature and pH on Fluorescence Values

Temperature

During earlier work (1, 2, 3) it was observed that temperature of extraction had an effect on the fluorescence value. The effect of temperature on both the fat and protein extractions was assessed by carrying out these operations at temperatures varying from 3.6° to 22.8° C., the remainder of the procedure being done at room temperature (23 to 25° C.). The results are given in Table V. The temperature coefficient for the defatting operation was 0.30

TABLE V

EFFECT OF TEMPERATURE OF FAT AND PROTEIN EXTRACTIONS ON FLUORESCENCE VALUES

Temperature, °C.	Fluorescence values	
	$CHCl_3$ extraction	KCl extraction
22.8	46.8	43.5
15.6	44.5	40.0
5.0	41.0	36.9
3.6	40.2	35.2

photofluorometer units per Centigrade degree, while that of the protein extraction was 0.35 units. Thus it may be seen that increase in temperature of 1° C. caused an over-all increase in the fluorescence value of 0.65 units. (1° F. change alters the value by 0.36 units.)

Day to day variations in laboratory temperature could, therefore, be expected to have some effect on the results. It is recommended that laboratory temperature be kept as nearly constant as possible, or failing this, that suitable corrections be made for temperature variations.

pH

It was previously noted that a relation existed between the pH of potassium chloride and water extracts of egg powder and the fluorescence value (3). To investigate this, the pH of aqueous extracts of fresh and stored egg powder was varied by means of hydrochloric acid and sodium hydroxide, and that of 10% potassium chloride extracts of similar stored powder with acetic acid and ammonium hydroxide. After the fluorescence at the altered pH values had been determined, an equivalent amount of acid or base was added to restore the pH to approximately its initial value, and the fluorescence again measured.

The results (Table VI) show that the fluorescence values varied with pH. Maximum values occurred in extracts of fresh powder at pH 2.5; in the stored powders, at pH 4.8 and 4.2. Between pH 1.0 and 10.0 the changes

TABLE VI

EFFECT OF CHANGING THE pH OF PROTEIN EXTRACTS ON FLUORESCENCE VALUES

Water extracts				10% KCl extract	
Sample No. 1		Sample No. 2		Sample No. 3	
pH	Fluorescence value[1]	pH	Fluorescence value[1]	pH	Fluorescence value[1]
(1 *N* acid)	27.5	(1 *N* acid)	27.5	(1 *N* acid)	67.6
1.21	11.2	1.20	32.9	3.26	35.0
2.48	25.2	2.51	47.0	3.83	49.4
3.36	17.7	3.88	51.3	4.18	57.9
4.29	11.5	4.82	73.8	4.39	47.0
5.42	11.2	6.19	47.5	5.05	37.0
6.92[2]	9.9[2]	8.15[2]	35.0[2]	6.29[2]	34.8[2]
7.23	10.3	8.18	35.4	9.88	34.8
9.36	11.0	9.78	27.7	10.12	34.8
				10.41	34.8
				10.60	32.8
				10.96	31.8
(1 *N* base)	7.0	(1 *N* base)	11.5	(1 *N* base)	67.4

[1] *Blank corrections for solvent applied (1).*
[2] *Extract without added acid or base.*

in fluorescence values were substantially reversible, e.g. the addition of an equivalent quantity of base to Extract 1 (Table VI) at pH 3.36 resulted in a fluorescence value of 10.0; the same extract containing the same amount of sodium chloride also had a fluorescence value of 10.0. However, reversibility was not obtained when extracts were made as strongly acidic or basic as 1 *N*.

From the foregoing, it might be assumed that variations in the pH of the water used in preparing the protein solvent might affect the fluorescence value. However, variations in the pH of the protein solvent from 4.6 to 8.9 had no significant effect on the fluorescence values of egg powders of widely varying quality (Table VII).

TABLE VII

EFFECT OF VARIATION IN pH OF SODIUM CHLORIDE SOLUTIONS ON FLUORESCENCE VALUES OF SIX SELECTED EGG POWDERS

pH of solvent	Mean fluorescence value
4.6	37.53
5.2	38.92
5.6[1]	38.65
8.9	39.45

[1] *pH of the distilled water used.*

Thus, altering the pH of the protein solvent has very little effect, whereas changing that of the extracts themselves does affect the values obtained. This behaviour might be explained on the basis of a "pseudo-buffering" effect of egg powder in protein solvents. It was found that egg powder with a pH of 7.13 in sodium chloride solution, when dispersed in sodium chloride plus sodium hydroxide solution with pH 8.50, assumed a resultant pH of 7.16. Therefore it seems unlikely that the range of pH ordinarily observed in laboratory distilled water will have any appreciable effect on the magnitude of the fluorescence value.

References

1. PEARCE, J. A. Can. J. Research, D, 21 : 98-107. 1943.
2. PEARCE, J. A. and THISTLE, M. W. Can. J. Research, D, 20 : 276-282. 1942.
3. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
4. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 194-202. 1943.
5. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 211-222. 1943.

INVESTIGATIONS ON THE USE OF IRISH MOSS IN CANNING OF MEAT[1]

By E. J. Reedman[2] and Leonard Buckby[3]

Abstract

In an attempt to develop a suitable substitute for the agar used in large quantities by the canning industry, gelose preparations were made from bleached and unbleached Irish moss. About 30 to 60% of the total moss solids, depending on the type of moss used, were extracted in one-half hour by hot water. Suspended solids were removed by filtration with diatomaceous earth, and the extracts purified with activated charcoal. Extracts were dried successfully on a laboratory model double-drum drier, an experimental spray drier, an experimental tunnel drier, and by air-drying the concentrated extract at room temperature. All methods of drying yielded products capable of forming good jellies.

Although agar jellies were definitely stronger than those from equivalent concentrations of Irish moss, the addition of 0.2% potassium chloride produced jellies from Irish moss that were stronger than those from agar. On the basis of consumer taste panel tests on canned chicken, no statistically significant difference could be demonstrated between the acceptability of agar and Irish moss jellies.

Introduction

The scarcity of agar due to wartime conditions has created a need for a substitute material. This is particularly true in the canning industry, which was one of the largest consumers of agar. In these circumstances algae other than the agar-producing species appeared to be worthy of investigation as possible sources of substitute materials.

Irish moss *(Chondrus crispus)* was chosen for study since it grows in large quantities in the waters of the Canadian east coast. It has already proved to be a valuable suspending and clarifying agent in several industries, either as an extract or the ground entire plant. The plant grows in shallow waters and is harvested from May to October by a raking process. Moss spread on the beach, allowed to dry and baled immediately is known as unbleached or black moss; bleached moss is allowed to lie exposed to the sun until a fairly uniform cream colour is produced. During the bleaching the moss is washed several times with sea water. Samples of such moss, from the east and west coasts of Prince Edward Island, were used in the present investigation, with the purpose of developing a method for the preparation of gelose from Irish moss and investigating the properties requisite for its use in canning of meat.

Procedure

Preparation of Sample

The type and origin of the moss samples used are indicated in Table I. Fresh moss was shipped wet to the laboratory, ground moss was produced by grinding whole air-dry plants in a Wiley mill through a coarse screen, and

[1] *Manuscript received July 6, 1943.*
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 101 of the Canadian Committee on Food Preservation and as N.R.C. No. 1151.

[2] *Biochemist, Food Investigations.*
[3] *Laboratory Assistant.*

TABLE I

YIELD AND PROCESSING DATA FOR IRISH MOSS EXTRACTIONS

Type, treatment, and origin of moss	No. of preparations examined	First extract				Second extract
		Conc., % solids	Soluble solids, % of total moss solids	pH	Viscosity at 100° F., time in sec. (Water = 34 sec.)	Soluble solids, % of total moss solids
Fresh moss						
Whole	1	0.41	50	7.73	36.8	10
Dried unbleached moss						
Whole (West Shore, Bag 1)	2	0.37	47	7.54	41.4	9
Ground (East Shore, Bag 3)	2	0.42	54	7.55	42.9	8
Leached (West Shore, Bag 1)	3	0.23	29	7.31	41.6	17
Dried bleached moss						
Whole (East Shore, Bag 2)	4	0.47	59	7.27	54.9	9
Ground (East Shore, Bag 2)	2	0.42	50	7.45	56.0	10
Leached (East Shore, Bag 2)	2	0.33	40	7.24	45.9	17

leached moss was prepared by washing in cold running water (below 5° C.) for three days. It was found that about 18% of the weight of unbleached moss was lost by leaching but only 3% of bleached moss was leached out. A considerable part of the weight lost by unbleached moss consisted of extraneous material such as sand. The bleached moss was invariably much cleaner.

Extraction

Since the gelose is readily soluble in hot water its extraction is not difficult. However, several methods were investigated. Only small amounts of gelling substances were extracted by cold dilute sodium hydroxide or cold dilute hydrochloric acid, and hydrolysis occurred in both instances when heat was applied.

Preliminary studies indicated that one-half hour extraction with hot water was the best practical procedure, further extraction being effected by increasing the proportion of water rather than by lengthening the time of extraction. However, further dilution adds to the difficulty of drying the extract. The extracts were prepared as follows: 150 gm. of the dry plant (containing 6 to 10% moisture) was extracted for one-half hour in 20 litres of water with constant stirring in a steam jacketed kettle. The temperature of the steam in the jacket was maintained at 100° C. The extracts were strained through a 30-mesh screen with stirring but without pressure. Further information was obtained by extracting the screened residue with another 20 litres of water for one hour and straining as before. This second extract was weighed, and after samples were taken for solids determinations, it was discarded.

The extract from leached moss was identical with the "hot extract" described by Haas and Hill (2).

Results listed in Table I show that the first extract contained soluble solids representing from 29 to 59% of the dry weight of original moss. Soluble solids in the second extract varied from 8 to 17% of the weight of the original moss. Data given are means of several extractions.

Filtration

The screened extract had undesirable colour, odour, and taste, and contained some suspended solid. Purification was accomplished by treatment with activated charcoal and filtration through diatomaceous earth. Since many of the extracts were quite viscous, difficulty in filtration set an upper limit on the amount of solids an extract might contain. Although unbleached moss gave filterable extracts at higher original concentrations than 150 gm./20 litres water, this was the maximum concentration that could be handled conveniently for bleached moss, and therefore was adopted as standard procedure throughout.

Purification entailed two steps: first, the removal of suspended solids; and second, the removal of colour, odour, and taste with activated charcoal. Attempts to remove all impurities by one filtration step proved impracticable with small-scale equipment. The filter press used was a four plate aluminum laboratory size model of standard design with eight filtering surfaces $4\frac{7}{8} \times 4\frac{7}{8}$ in. Canvas duck was used for press cloths.

The procedure finally adopted was as follows: the filtering surfaces were given a precoat of 0.15 lb. coarse filter aid per square foot of filtering surface; 1% of the filter aid was mixed with the extract and the mixture then passed from a blow-case through the press under 40 to 50 lb. air pressure. The extract was kept hot by means of a steam jacket in the press. To the filtered extract was added 0.5% of activated powdered charcoal and the mixture stirred for one-half hour. The press was then cleaned and given a precoat similar to the first but using a finer filter aid. One per cent of the filter aid was also added to the extract which was then passed through the press as before. The filtrate was dried to yield solid preparations for test purposes.

All odour and taste were removed from all types of samples except fresh moss that had undergone some bacterial decomposition before reaching the laboratory. Most of the colour was also removed, but only the extract of leached, bleached samples could be called colourless. The extracts were progressively less coloured in this order: unbleached, leached unbleached, bleached, and leached bleached. It is realized that the figures given for concentrations of extract and quantities of filter aid and charcoal will not necessarily be suitable for industrial use.

Drying

Excessive heat applied to dry moss or solid extract produced hydrolysis. Nevertheless it was found that almost any method of drying could yield a product capable of forming good jellies. Since, as shown in Table I, the extracts contained only 0.23 to 0.47% solids, drying presented some difficulty. However, extracts were dried successfully on a laboratory model double-drum drier, an experimental spray drier, an experimental tunnel drier, and by concentrating the extract, allowing it to set, and drying small blocks of the resultant jelly with a fan at room temperature.

Extracts were fed to the drum drier from an overhead tank through tubes that ran the width of the drums. The drums were heated by steam at 50 lb. pressure. Since the knives could cut off the dry material only when it had attained a certain thickness on the drum, the material from dilute extracts remained on the drums longer than that from more concentrated extracts, and charring sometimes took place. Extracts preconcentrated to about 3% solids were dried successfully, although they were too viscous to be fed to the drums from the tubes described above. The dried product consisted of curled strips of solid and on grinding in a Christie mill yielded a powder of light brown colour.

Spray-drying produced the most attractive looking solid—a light, pure white powder, but this product was difficult to redissolve.

Extracts were tunnel-dried in galvanized sheet-iron trays with $\frac{3}{4}$ in. sides. A layer $\frac{1}{2}$ in. deep dried in four hours at 93° to 107° C., giving thin translucent sheets of solid that were slightly charred in spots. The ground product was similar in appearance to drum-dried solid.

Preconcentration of material for drying at room temperature required heating of the extract by steam at 100° C. in a jacketed open kettle for about four hours. The extract was constantly stirred mechanically and a stream of air was passed over its surface. As the extract was concentrated, some solid dried on the side of the kettle, and was collected (kettle-dried). The extract after preconcentration was allowed to set, cut into blocks about 2 in. × 2 in. × 1 in. and dried by a fan at room temperature for about 36 hr. Case-hardening always occurred. The ground product was similar in appearance to drum-dried and tunnel-dried material.

A method of preparing a pure gelose from Irish moss by precipitation with ethanol has been described (1). Owing to the large quantities of ethanol necessary to produce small amounts of dried gelose, this method is more suited to laboratory than to industrial use. Dry solid was prepared by precipitation with ethanol and also with acetone. The extract was concentrated to about 2 to 3%, divided into two equal portions, one portion poured with stirring into three times its volume of 95% ethanol and the other into a similar volume of acetone. The precipitate was removed as it formed, surplus liquid removed by squeezing, and the resulting material placed in sufficient fresh ethanol (95%) or acetone to cover it. After stand-

ing overnight the precipitates were removed from the cover-liquor, dried for 24 hr. at room temperature in slowly moving air, and then for two to three hours in an air-oven at 75° C. Approximately equal weights of solid were yielded by the two precipitants. The dried material was ground in a mortar and was then intermediate in appearance between spray-dried and drum-dried solids. Its ease of solution was intermediate also, indicating that with finer division of particles the dried extract was lighter in colour but more difficult to dissolve.

TABLE II

ANALYSES OF SAMPLES OF IRISH MOSS AND OF DRIED EXTRACTS PREPARED FROM IRISH MOSS BY VARIOUS METHODS

Type and treatment of moss	Method*	Moisture, %	Moisture-free basis		
			Ash, %	Chloride, %	Nitrogen, %
(a) Samples of Irish moss					
Unbleached moss					
Ground	—	8.0	29.7	1.37	1.20
Leached	—	10.4	21.8	0.21	1.63
Bleached moss					
Ground	—	9.6	22.9	0.14	1.00
Leached	—	11.1	19.2	0.06	0.93
(b) Dried extracts prepared from Irish moss					
Unbleached moss					
Whole	1. Drum	7.0	26.0	0.13	0.27
	3. Tunnel	2.6	30.0	3.72	0.27
Ground	2. Drum	5.1	37.8	6.72	0.27
	3. Tunnel	4.1	31.8	3.72	0.26
	4. Ethanol	11.2	24.6	0.06	0.20
	5. Acetone	11.2	27.5	0.06	0.22
Leached	2. Drum	9.3	23.5	0.08	0.08
	3. Tunnel	7.8	25.2	0.05	0.14
	4. Ethanol	6.8	19.8	0.08	0.11
	5. Acetone	6.6	21.1	0.06	0.11
Bleached moss					
Whole	1. Drum	7.8	25.0	0.09	0.15
	3. Tunnel	7.3	27.2	0.07	0.21
Ground	2. Drum	8.8	29.0	0.28	0.17
	3. Tunnel	9.5	29.7	0.29	0.16
	4. Ethanol	5.1	22.2	0.05	0.14
	5. Acetone	4.1	23.8	0.05	0.14
Leached	2. Drum	11.4	21.5	0.07	0.08
	3. Tunnel	7.1	23.4	0.02	0.14
	4. Ethanol	7.7	19.7	0.04	0.09
	5. Acetone	7.4	20.8	0.02	0.09

**Processes are described in text.*

The five methods of preparation used are listed in Table II. In Method 1 the air-dry moss, as received, was weighed into a 30-mesh screen, soaked in cold water for 10 min., and the filtered extract from this preparation drum-dried as described. The 10 min. soaking was omitted in the remaining

methods, the filtered extract being drum-dried in Method 2, tunnel-dried in Method 3, concentrated and precipitated with ethanol in Method 4, and concentrated and precipitated with acetone in Method 5.

Examination of Dried Extract

One gram samples were dried overnight in an air-oven at 98° C. to determine moisture and subsequently were ashed in an electric muffle furnace for 24 hr. at 550° C. Chlorides in the ash were determined by the Mohr method. Nitrogen determinations were made on 1 gm. samples by the Kjeldahl method. The results are shown in Table II.

TABLE III

EFFECTS ON JELLY STRENGTH OF ADDING VARIOUS SALTS TO 1% AGAR AND 2% GELS OF IRISH MOSS

Description of sample	Salt added, %	Second jelly strength, gm. Hg.	Appearance of final jelly
2% agar	None	41.6	Opaque
1% agar	None	8.2	Fairly opaque
	Sodium chloride, 0.1	—	Sample lost
	Calcium chloride, 0.1	3.3	Fairly opaque
	Ferric chloride, 0.1	0	Hydrolysed
Unbleached moss, leached, drum-dried	None	3.6	Clear
	Sodium chloride, 0.1	0	Viscous liquid
	Calcium chloride, 0.1	0	
	Ferric chloride, 0.1	0	Hydrolysed
Unbleached moss, ground, acetone precipitated	None	8.3	Slightly opaque
Unbleached moss, ground, ethanol precipitated	None	0	Clear
	Ash* ≈ 0.5	45.9	Opaque
Mixture of various extracts, No. 1	None	0	
	Sea salt ≈ 0.4	4.7	Slightly opaque
	Magnesium chloride, 0.1	0	
	Magnesium sulphate, 0.1	0	
	Potassium bromide, 0.1	5.2	Opaque
	Calcium sulphate, 0.1	0	Slightly opaque
	Calcium carbonate, 0.1	0	
	Potassium sulphate, 0.1	11.0	Opaque
Mixture of various extracts, No. 2	None	2.8	
	Potassium sulphate, 0.1	13.2	Slightly opaque
	Potassium carbonate, 0.1	20.3	Opaque, white ppt.
	Rochelle salt, 0.01	3.7	
	.1	5.4	
	Potassium chloride, 0.01	4.2	
	.025	5.9	Slightly opaque
	.05	8.4	
	.1	14.1	
	.2	33.7	
Bleached moss, ground, drum-dried	Potassium chloride, 0.2	62.9	
	.3	77.0	
	.4	95.8	Clear, ppt.
	.5	104.8	

* *Ash from preceding sample.*

Examination of Jellies

Since one of the criteria of the usefulness of any preparation was its ability to form a firm jelly, 2% solutions of each were prepared and the comparative strengths of the jellies determined. The instrument used for determining jelly strengths was constructed in these laboratories and has been described elsewhere (4).

Jellies were prepared as follows: 2 gm. of dried extract was weighed into 7-oz. flat sanitary cans, 100 ml. of boiling, distilled water was added, and the mixture stirred on a steam-bath for two minutes. The cans were then closed, retorted for 10 min. at 121° C. and cooled with running water. Jellies made with distilled water (Table IV) were not cooled after retorting; consequently

TABLE IV

STRENGTH OF JELLIES FROM 2% IRISH MOSS GELOSE, WITH AND WITHOUT ADDED POTASSIUM CHLORIDE

Type of moss used and method of drying extracts	With distilled water			With addition of 0.2% potassium chloride		
	First jelly strength, gm. Hg	Second jelly strength, gm. Hg	Appearance of final jelly	First jelly strength, gm. Hg	Second jelly strength, gm. Hg	Appearance of final jelly
Fresh, wet moss						
Whole, drum-dried	28.9	29.3	Fairly opaque	31.8	57.4	Fairly opaque
Fresh, dried moss						
Whole, drum-dried	39.2	32.7	Opaque	34.5	54.8	Fairly opaque
Unbleached moss						
Whole, air-dried	17.4	22.1	Slightly opaque	25.6	39.4	Slightly opaque
drum-dried	9.5	9.4	Clear	24.2	51.0	Clear, dark ppt.
tunnel-dried	22.7	28.7	Fairly opaque	45.0	48.8	Opaque
Ground, kettle-dried	12.6	0	Clear, ppt.	—	—	—
drum-dried	19.4	22.2	Clear	31.5	42.1	Clear, dark
tunnel-dried	16.7	7.7	Slightly opaque	28.9	40.4	Fairly opaque
ethanol precipitated	11.9	0	Clear	36.7	52.0	Slightly opaque
acetone precipitated	11.5	5.8	Clear	36.8	55.7	Slightly opaque
Leached, kettle-dried	6.3	0	Opaque	—	—	—
drum-dried	3.6	0	Slightly opaque	22.1	42.6	Opaque
tunnel-dried	4.0	0	Opaque	45.4	41.9	Opaque
ethanol precipitated	5.9	0	Opaque	43.0	78.6	Fairly opaque
acetone precipitated	6.4	0	Opaque	32.1	73.0	Fairly opaque
Bleached moss						
Whole, air-dried	12.2	5.1	Clear	9.6	41.8	Slightly opaque
drum-dried	2.6	0	Clear	32.2	52.4	Slightly opaque
tunnel-dried	6.7	0	Slightly opaque	47.1	38.4	Slightly opaque
Ground, kettle-dried	6.4	0	Slightly opaque	45.7	52.9	Slightly opaque
drum-dried	7.4	0	Clear	53.3	62.9	Clear
tunnel-dried	7.9	0	Opaque	45.9	52.3	Opaque
ethanol precipitated	8.6	0	Clear	38.5	54.1	Slightly opaque
acetone precipitated	9.6	0	Slightly opaque	33.6	59.8	Slightly opaque
Leached, kettle-dried	5.4	0	Opaque	42.0	79.8	Opaque
drum-dried	4.0	0	Slightly opaque	82.7	81.6	Fairly opaque
tunnel-dried	6.8	0	Slightly opaque	28.8	27.6	Opaque
ethanol precipitated	13.1	0	Slightly opaque	67.2	91.4	Fairly opaque
acetone precipitated	22.6	0	Slightly opaque	77.9	90.0	Fairly opaque

they received more severe heating treatment than other preparations. The 10 min. retorting was in some instances insufficient to dissolve the dried extract completely, as evidenced by lumps of more concentrated mixture throughout the gel. This accounts for such discrepancies as greater jelly strengths for the longer retorting period (Table IV). The measure described in Table IV as first jelly strength was taken after the gels from the 10 min. retorting had stood overnight to come to room temperature (about 24° C.). After this measurement was made the cans were heated at 100° C. for five minutes and the contents transferred to new cans which were closed and retorted for one hour at 121° C. The second jelly strength measure was then made on these gels after standing overnight.

Addition of Potassium Chloride

Results in Table IV indicated that preparations from unbleached moss formed stronger jellies than those from bleached moss. It was also observed that leaching—in fact even the 10 min. soaking process—removed something from the extract that would otherwise have contributed to jelly strength. The fact that this substance was removed by the 10 min. soaking pointed to its being very soluble in water, suggesting a salt. Strengthening effects of alkali and alkaline earth salts on Irish moss jellies have been reported (3).

Various amounts of sodium, calcium, and ferric chlorides were added during the preparation of certain extracts. The only noticeable effect was the hydrolysis produced by ferric chloride (Table III). Additions of ash from preparations giving good jellies to preparations giving poor jellies resulted in jellies stronger than those yielded by the materials from which the ash was taken.

Sea water was used instead of distilled water in one preparation and the resulting jelly was stronger. A series of jellies was then made up with the addition of each of the various salts occurring in sea water (Table III). Potassium bromide was substituted for magnesium bromide, which was not on hand at the time. The results showed that only the potassium salts gave jellies of increased strength, and that jelly strength increased approximately in proportion to the amount of potassium added. This was particularly true of the jelly containing potassium carbonate which, however, contained considerable white precipitate, probably calcium carbonate. The jelly strength fell off slightly with Rochelle salt, potassium sulphate, and potassium chloride, in that order. However, potassium chloride was chosen as most economical with respect to its potassium content per unit weight, and jellies were made from all preparations with addition of this salt.

Table III shows that over the range of concentrations used, jelly strength increased in a straight line relationship with addition of potassium chloride. It is obvious that all added potassium is not taken up into the gelose structure. A potassium salt of the gelose prepared by dialysis has been reported to contain 12% potassium (1), but even 0.5% potassium chloride in these materials represents approximately 13% potassium with respect to gelose.

The effect of adding potassium chloride to preparations of the gelose was generally to equalize the strengths of resulting jellies. The jellies of highest strengths were those made from the preparations that were purest as shown by ash content; i.e. those made by precipitation with ethanol and acetone. The difference in jelly strength between leached bleached drum-dried and leached bleached tunnel-dried preparations may be accounted for on the basis of hydrolysis due to excessive heating in drying. This factor while not noticeable in the remaining data, may have had some effect.

While gelose from Irish moss appeared to go into solution at a lower temperature than agar, at a higher temperature agar went into solution more readily. During the stirring on the steam-bath, prior to closing the cans, part of the Irish moss gelose dissolved but the rest caked into small lumps throughout the liquid. Agar, however, in this stage, did not go into solution but was evenly dispersed throughout the liquid. After 10 min. retorting, agar had gone entirely into solution but lumps of undissolved Irish moss gelose still persisted. For this reason, comparisons between agar and Irish moss first jelly strengths (Table V) are not valid. Since both agar and Irish moss were completely dissolved at the end of the one hour retorting period,

TABLE V

STRENGTH OF JELLIES FROM AGAR AND IRISH MOSS GELOSE OF VARYING CONCENTRATION, WITH AND WITHOUT ADDED POTASSIUM CHLORIDE

Material tested	Concentration of gelose, %	With distilled water			With addition of 0.2% potassium chloride		
		First jelly strength, gm. Hg	Second jelly strength, gm. Hg	Appearance of final jelly	First jelly strength, gm. Hg	Second jelly strength, gm. Hg	Appearance of final jelly
Agar	0.5	4.4	3.2	Slightly opaque			
	1.0	10.9	6.9	Fairly opaque			
	1.5	23.7	15.9	Opaque			
	2.0	41.0	27.6	Opaque	41.6	32.2	Opaque
	2.5	56.2	45.7	Opaque			
	3.0	79.3	63.1	Opaque			
Unbleached whole moss extract, air-dried at room temperature	0.5	0	0	Clear	5.1	4.5	Clear
	1.0	3.8	3.6	Clear	13.0	11.1	Clear
	1.5	7.0	7.8	Clear	22.8	19.5	Slightly opaque
	2.0	12.1	15.9	Clear	30.2	31.5	Slightly opaque
	2.5	22.4	26.8	Slightly opaque	41.8	49.6	Quite opaque
	3.0	24.1	38.0	Slightly opaque	42.7	59.9	Opaque

the second jelly strengths are comparable. Table V shows a series of Irish moss gelose, with and without added potassium chloride, and an agar series, at concentrations of from 0.5 to 3%. Although agar jellies were definitely stronger than those from equivalent concentrations of Irish moss when no potassium chloride was added, addition of this salt produced jellies from Irish moss that were stronger, in the main, than those from agar.

Hydrogen ion concentrations in jellies from Irish moss over a range from pH 8 to pH 6 apparently had no effect, but there appeared to be a gradual dropping off in strength below pH 6 (Table VI).

TABLE VI

EFFECT OF VARYING pH ON JELLY STRENGTH OF 2% GELOSE FROM UNBLEACHED WHOLE MOSS EXTRACT, AIR-DRIED AT ROOM TEMPERATURE

pH	Second jelly strength, gm. Hg	Appearance of final jelly
7.93	20.8	Slightly opaque
7.13	19.8	
6.13	20.0	
5.06	12.5	
4.15	4.0	

Taste Panel Tests

Since the purpose of the study was to investigate the usefulness of Irish moss gelose as a gelling agent with canned meat, an experimental pack of chicken was put up in 7-oz. cans. The chickens used were Grades *A* and *B* Milk Fed birds that had been in storage at −40° C. for over a year and had suffered some desiccation. An open precook of one hour was used after which the meat was removed from the bones and pooled. Ten cans were packed with 3½ oz. of meat, half light and half dark, taken at random from the whole supply. The broth was divided, salt and 2% agar were added to one-half and salt and 2% Irish moss gelose to the other. Three and one-half ounce portions of each of these broths were added to five cans containing meat. The cans were than closed and retorted for 45 min. at 121° C.

A panel of 24 tasters was asked to score samples of the product, with particular attention to the jelly, on a taste scale ranging from 10 for excellent to 0 for inedible. Under these conditions, a preference for agar of 0.75 score unit was shown by the tasters but this was below the level of significance ($t = 1.72$). It would therefore appear that Irish moss gelose has distinct possibilities as a substitute for agar. The work is continuing.

Acknowledgments

Grateful acknowledgment is made to Dr. A. W. H. Needler, Director, Atlantic Biological Station, Fisheries Research Board of Canada, through whose aid samples of moss were obtained. The authors also wish to acknowledge their indebtedness to the late James Pike, Chief Engineer, *S.S. Caribou*, who provided the sea water used in these studies.

References

1. BUTLER, M. R. Biochem. J. 28 : 759-769. 1934.
2. HAAS, P. and HILL, T. G. Ann. Applied Biol. 7 : 352-362. 1921.
3. LÉON, J. H. Fr. 680,188, Aug. 12, 1929.
4. REEDMAN, E. J. Can. J. Research, D, 21 : 324-331. 1943.

THE IDENTIFICATION OF WIREWORMS OF ECONOMIC IMPORTANCE IN CANADA[1]*

By Robert Glen[2], Kenneth M. King[3], and A. P. Arnason[4]

Abstract

Over 30 different wireworm types are separated in the key presented. Their ecology and economics are discussed briefly and reference is given to the most important literature on biology, morphology, and control. Although descriptive morphological details are omitted, the separating characters given are derived from extensive detailed studies.

Perhaps in no field of economic entomology has progress been more retarded by lack of recognition of the species involved than in that dealing with wireworms. Although wireworm investigators have recognized the species with which they were working, other entomologists all too often have seemed to regard wireworms as constituting only a single economic form. In reports and textbooks especially, the subject has been treated very inadequately, reference usually being made simply to "wireworms". This has created the false impression that all wireworms are alike and that control measures are universally the same. As a result many data on habits, distribution, and control are of little value because of their generality.

The Preparation of Simple Field Keys

The primary objective of this paper is to provide basic information from which field men throughout Canada can prepare simple keys for the identification of the wireworm pests in their localities. The preparation of such keys is not difficult. A beginning can be made from the data here presented, to be supported ultimately by a local reference collection of reliably identified wireworms.

As a first step, a list of the pest species now known to occur in a particular province may be prepared by consulting Page 374. From the discussions in the text, these species may be further grouped on the basis of common habitats, viz., muck soil, parkland, open prairie country, etc. Examination of the illustrations and text will reveal the structural differences of the wireworms found in the same habitat. These gross differences of habitat and structure may then be integrated into a preliminary field key. The great majority of species that occur together in the same habitat are so distinct morphologically that a hand lens suffices for their identification, once the separating features are known.

* *An abstract of the main ideas embodied in this paper was prepared in 1938 (Reference 21).*

[1] *Manuscript received June 29, 1943.*
Contribution from the Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada. Contribution No. 2248.

[2] *Assistant Entomologist.*
[3] *Entomologist-in-Charge.*
[4] *Assistant Entomologist.*

An alternative or supplementary method is to develop the key coincident with the collecting of material. As soon as two different species are obtained, a simple key may be made for their separation. With further additions to the collection, the key should be expanded and amended until it includes all reliably named local forms.

The local key should not be merely a fraction of the "master key" here presented. In a key to all of the economic wireworms in Canada, it is necessary to make use, at times, of minute and relatively difficult characters. However, most practical field keys need include only gross structural differences. The identification of the larvae of *Ludius aeripennis destructor* and *Cryptohypnus nocturnus*, which occur together in western grain fields, serves as an example. To run these larvae through the master key would entail examination of many different structures, whereas reliable separation is possible by using a hand lens to examine the ninth abdominal segment (Figs. 45, 46 vs. 57, 53).

Regardless of how the local key is derived, a reliably named reference collection should be built up as soon as possible. The suggested procedure is to forward all unfamiliar wireworms to specialists for identification. The Saskatoon Laboratory is now in a position to offer assistance, both in making identifications and in supplying specimens of the common pest species.

Important Identifying Characters

In identifying wireworms the final decision must be based largely upon morphology, although information on habitat and habits has proved repeatedly to be of great assistance. Facility in using structural characters, as presented in the master key, depends primarily upon the following: (*a*) a working knowledge of the general morphology of elaterid larvae; (*b*) the reliability of the various taxonomic characters used; and (*c*) the common structural differences between young and mature larvae of the same species. These are discussed in the paragraphs that follow.

Although elaterid larvae are quite diverse in form (Figs. 1 to 11) they can be readily distinguished from larvae of other families occurring with them in farm land by the following combination of characters: three pairs of thoracic legs, well-developed, subequal in size, five-segmented (counting the terminal claw as a segment); labrum absent or fused with clypeus and anterior margin of frons into a rigid nasale (*n*, Fig. 12; Figs. 15 to 19); frontoclypeal area usually lyre-shaped (*fcl*, Fig. 12); maxillae and labium elongate, and fused into a single unit (*vmth*, Fig. 13); body straight, with nine abdominal segments visible dorsally, the ninth with or without paired terminal processes (= urogomphi); the 10th abdominal segment bears the anus and lies ventrad to the ninth and may or may not be armed by sclerotized tubercles or hooks; spiracles biforous.

False wireworms (Tenebrionidae), mainly of the genus *Eleodes*, bear superficial resemblance to some of the true wireworms of the subfamily Elaterinae. However, separation is relatively simple since false wireworms possess a well-

developed true labrum (*lbr*, Fig. 14), a Y-shaped frontal suture (*fs*, Fig. 14), more conspicuous antennae (*ant*, Fig. 14), an upturned tip and marginal spine-like setae on the ninth abdominal segment (Figs. 35, 41), and, in many species, enlarged prothoracic legs.

As far as possible, the most dependable characters have been selected for the key. Nevertheless, these are subject to some individual variation much of which is caused by "erosion"—a term applied to changes that occur during an instar as a result of feeding or movement through the soil. Structures that are subject to erosion are best observed on recently moulted specimens, since their taxonomic value is progressively reduced as the larva approaches the end of each instar. It is recommended that workers become familiar with the more important diagnostic structures and their relative dependability, from the following discussion.

The ninth abdominal segment (*9*, Figs. 1, 2, 7) is the most important structure in the identification of wireworms to genus and species. If lacking a median caudal notch: the tip of the segment may be flattened (Figs. 30, 31), smoothly rounded (Fig. 38), bluntly pointed (Figs. 33, 34, 40), or sharp (Figs. 32, 36, 37); the tergum may bear "eye spots" (*es*, Fig. 33) or whorls of preapical setiferous tubercles (*tub*, Figs. 36, 37, 40) or lack these; the sternum may be large (*st*, Figs. 34, 36, 47) or small. If possessing a median caudal notch: the notch, relative to the size of the segment, may be large (*cn*, Figs. 43, 45, 49) or small (*cn*, Figs. 58, 62); the urogomphi divided (*ur*, Figs. 44, 45, 49) or undivided (Figs. 42, 43), short and thick (Figs. 51, 53, 55) or relatively long and slender (Figs. 52, 54), with urogomphal prongs of various shapes; the dorsum of the segment may bear setae in the central area (Figs. 45, 50, 55) or lack such setae (Figs. 49, 57), and the lateral margins may be smooth (Fig. 64) or have protuberances that are blunt (*to*, Figs. 57, 62) or prominent, sharp and tooth-like (*to*, Figs. 47, 54). These characters are highly dependable. Erosion may dull and shorten the urogomphal prongs and some of the tooth-like protuberances, but the general character of the segment usually remains.

The nasale (*n*, Fig. 12) is a very useful structure, although usually requiring microscopic examination. It may be absent, or present as several teeth (Fig. 15) or as one tooth terminating in a single point (Fig. 16) or triple points (Figs. 17, 18, 19). It is subject to considerable erosion, but in the great majority of larvae the general type of nasale can be recognized even when quite worn. The nasale loses much of its diagnostic value when it is of a type having very small lateral denticles, which are commonly worn off.

Mandibles (*md*, Fig. 12) provide good separating characters, visible when fully opened. They may lack teeth on the inner aspect (Fig. 20), or possess one tooth, the "retinaculum" (*ret*, Fig. 22), or have teeth in addition to the retinaculum (Figs. 21, 23). Erosion may dull and shorten the points, but only minute denticles and ridges are likely to be completely worn away.

Prominent sculpturing, such as impressions (*im*, Figs. 26, 29, 30) and deep pits (Fig. 32), is an excellent supplementary character. The length of impressions and the density of pits frequently vary considerably between indi-

viduals of the same species and therefore are relatively unreliable characters for the separation of species in which the differences in sculpturing are slight. Sculpturing is less conspicuous on very immature larvae and on recently moulted specimens.

The presternum of the prothorax is a large triangular anterior area that may be composed of several pieces (*prst*, Fig. 27) or united into one sclerite (Fig. 24). The divided type is sometimes difficult to observe clearly in small larvae or in badly shrunken specimens. However, the character is of sufficient taxonomic importance to warrant practice in its use.

Eyes present or eyes absent is an excellent supplementary character. However, a binocular is required to observe the eyes and familiarity with their normal position is important. When present, the eye will be seen as a single, small, ovate, dark spot (under the integument) on the side of the head, situated a short distance posterior to, and in line with, the lower margin of the base of the antenna. One or more prominent setae usually occur just above the eye. The presence of eyes is more readily detected in fresh specimens. In prepupal or premoult larvae the eyes may be displaced. However, in species that have eyes the integument is not pigmented in the region normally occupied by the eye and the presence of a clear spot at the appropriate location may be taken as evidence of eyes being present.

Setae often vary markedly both in numbers and in arrangement. Contrasts, such as setae absent or setae present, are reliable differences, but as a rule setal characters should be supported by other evidence.

Size must be used with care, because the measure of length of a given specimen might vary considerably, depending upon the degree to which the specimen is distended. It is a useful supplementary character for unusually small or unusually large species.

Colour cannot be relied upon except between marked contrasts such as pale yellow and dark brown. Recently moulted specimens are always pale and for a time may lack characteristic colouring.

Biological characters. Little is known of the bionomics of most pest species and every opportunity should be taken to increase our knowledge. Information on habitat, life-history, and feeding habits often provides valuable clues to identification and very frequently is an essential complement to a relatively unreliable or obscure structural character. Sometimes all of the known larvae of a genus have similar habits. In such larvae, biological characters are of generic value. In other groups, the biology is diverse and difference in habit is specific. Some genera appear to be entirely predacious, and in many genera of economic importance there are several non-economic species living in wood or leaf litter. Such forms sometimes are encountered in fields of recently broken wooded land. Excellent reviews of the literature on biology and control of wireworms have been provided by Thomas (56, 57).

The characters used in the key were taken from well grown larvae. If younger specimens are examined slight differences may be expected. Young larvae usually differ from mature larvae of the same species by being some-

what paler in colour and by having fewer setae, tubercles, and denticles, and less conspicuous sculpturing. First instar larvae may differ markedly, especially in characters of nasale, caudal notch, and urogomphi. The number of larval instars of many elaterids varies so widely that it is not possible to determine the relative maturity of a given specimen. However, the key characters presented are known to hold for all larvae that are half grown or larger.

KEY TO WIREWORM PESTS IN CANADA†

1. Without a median caudal notch on ninth abdominal segment (Figs. 1 to 4)...(Subfamilies Cardiophorinae and Elaterinae)....2
   With a median caudal notch on ninth abdominal segment (*cn*, Figs. 5 to 11)...(Subfamilies Oestodinae and Pyrophorinae)....10
2. Abdomen with pseudo-segmentation (Fig. 1); larva extremely slender; mandible deeply cleft into ventral and dorsal branches. Widely distributed in sandy and light loam soils...(Subfamily Cardiophorinae)....**Cardiophorus* spp.
   Abdomen with normal segmentation (Figs. 2 to 11); mandible not of above type..(Subfamily Elaterinae)....3
3. Tip of ninth abdominal segment somewhat flattened and scalloped (Figs. 30, 31); usually with striate impressions anteriorly on dorsum of each abdominal segment (*im*, Figs. 4, 30); nasale of one single-pointed tooth (as in Fig. 16); eyes absent. Southern Manitoba and Eastern Canada....*Melanotus* spp.
   Tip of ninth abdominal segment not flattened (Figs. 34, 36)....4
4. Tip of ninth abdominal segment smoothly rounded (Fig. 38); sternum of ninth abdominal segment greatly reduced. Inhabiting decaying wood....**Parallelostethus* and related genera
   Tip of ninth abdominal segment pointed, either bluntly (Fig. 33) or sharply (Figs. 32, 37); sternum of ninth abdominal segment well developed (*st*, Figs. 34, 36)....5
5. With striate impressions and conspicuous pits on dorsum of abdominal segments (Fig. 32); nasale of one single-pointed tooth (as in Fig. 16). Widely distributed; in decaying wood....**Ampedus* spp.
   Without striate impressions or conspicuous pits on abdominal segments; nasale of one triple-pointed tooth (Fig. 19)....6
6. Two "eye spots" (conspicuous pigmented areas) near front of ninth abdominal segment (*es*, Fig. 33); mandible with tooth-like expansion of dorsal cutting edge (*to*, Fig. 23). Manitoba and Eastern Canada; generally in poorly drained fields and associated with grass....*Agriotes mancus*
   Without "eye spots" on ninth abdominal segment; mandible without expanded dorsal cutting edge (Fig. 22)....7
7. With small preapical setiferous tubercles‡ on ninth abdominal segment (*tub*, Figs. 36, 37, 40)....9
   Without such tubercles (Figs. 34, 39)....8
8. Ninth abdominal segment with blunt tip (Fig. 34). Widely distributed in park and forest areas....*Agriotes limosus*
   Ninth abdominal segment terminating in small nipple-like point (Fig. 39). Larva known only from Pemberton, B.C....Unidentified, probably *Agriotes* sp.
9. Ninth abdominal segment (Fig. 40) with blunt tip and one or two whorls of minute preapical tubercles. Southwestern Saskatchewan and southern Alberta; in open prairie country....*Agriotes criddlei*
   Ninth abdominal segment (Figs. 36, 37) with sharp tip and two or three whorls of small preapical tubercles. Widely distributed; usually in parkland or well-watered soils....*Dalopius* spp.

† *As an aid to the accurate identification of the economic species, characters are included for the separation of the common non-economic types encountered in soil and leaf litter. Species that have not been identified through rearing are shown by a question mark; non-economic forms are indicated by an asterisk.*

‡ *These tubercles may be very minute and easily overlooked by an inexperienced observer. They are merely slight elevations (usually more deeply pigmented) of the body wall around the bases of certain setae. Their presence is most readily detected by comparing the bases of setae that are situated well forward on the segment with those near the tip. Where the setal bases are similar throughout the segment, "setiferous tubercles" are lacking.*

10 (1). With two large, horny prongs issuing from dorsum of ninth abdominal segment (*dpr*, Fig. 5); nasale absent or greatly reduced... (Subfamily Oestodinae).....11
Without horny prongs in front of urogomphi (Figs. 6 to 11); nasale well-developed... (Subfamily Pyrophorinae).....12

11. Saskatchewan and Alberta; usually in alkaline situations.....*Oestodes puncticollis*
Eastern Canada and Manitoba.....*Oestodes tenuicollis*

12. Urogomphi undivided (Figs. 42, 43).....13
Urogomphi divided (Fig. 44), although sometimes outer prong is greatly reduced (*opr*, Figs. 45, 58, 64).....14

13. Urogomphi blunt, directed backward (Fig. 42); nasale of one single-pointed tooth (as in Fig. 16); eyes present. Widely distributed through northern forest; in leaf litter.....
.....**Eanus decoratus*
Urogomphi pointed, incurving (Fig. 43); nasale of three short inconspicuous teeth; eyes absent. Prairie Provinces; in lighter soils of open prairie land.....*Hypnoidus dubius*

14. Without teeth on inner aspect of mandible (Fig. 20); head and prothorax reddish-brown, abdomen pale yellow and soft (see Fig. 6); nasale of three sharp teeth (Fig. 15); "anal armature" on 10th abdominal segment (*ar*, Fig. 47); ninth abdominal segment flattened, with prominent, sharp protuberances on lateral margins of dorsum (Figs. 44, 47). Ontario and Western Canada.....*Aeolus mellillus*
With teeth (usually only one tooth) on inner aspect of mandible (*ret*, Fig. 22); larva without contrasting colours on dorsum; nasale of one tooth, either single-pointed (Fig. 16) or triple-pointed (Figs. 17, 18, 19); without anal armature.....15

15. With large caudal notch on ninth abdominal segment (*cn*, Figs. 45, 49, 50, 54, 55, 56, 57), the posterior aperture of notch being wide.....16
With small caudal notch (*cn*, Figs. 58, 59, 60, 62, 63, 64) the posterior aperture of notch being narrow or entirely closed.....26

16. Outer prongs of urogomphi small, erect (*opr*, Figs. 45, 46); caudal notch U-shaped, with sides subparallel (*cn*, Fig. 45). Widely distributed; important crop pest on grass plains and parklands of Prairie Provinces.....*Cryptohypnus nocturnus*
Prongs of urogomphi subequal in length (Figs. 49, 50, 54, 55, 56, 57).....17

17. †With one small seta laterad to, but near each side of posterior part of frons (*ped*, Figs. 8, 25) and on anterior part of each body segment, as figured (*atm*, Figs. 8, 25); four setae on central dorsal area of ninth abdominal segment, the posterior setae being larger (Fig. 50); nasale of one triple-pointed tooth (lateral denticles sometimes worn off).....18
Without setae *ped* and *atm* (Figs. 7, 9, 10); with one of the following combinations of characters: (i) setae present on central dorsal area of ninth abdominal segment, nasale single pointed; or (ii) setae absent on above area, nasale single pointed; or (iii) setae absent on above area, nasale triple pointed.....19

18. Tip of outer prong of urogomphus curving backward (*opr*, Fig. 48); transverse branch of impressions on dorsum of first to eighth abdominal segments reaching approximately one-half distance to mid-dorsal line (as in Fig. 8). Eastern Saskatchewan, Manitoba, and Eastern Canada; usually in poorly drained soils.....*Cryptohypnus abbreviatus*
Tip of outer prong curving forward; transverse branch of impressions reaching to or nearly to mid-dorsal line on second to eighth abdominal segments (as in Fig. 7). In forested areas; in leaf litter.....**Ludius* spp.

19. One large triangular sclerite comprising presternum of prothorax (*prst*, Fig. 24); nasale of one triple-pointed tooth; sometimes with conspicuous punctures on dorsum of ninth abdominal segment (Fig. 49); other abdominal segments conspicuously sculptured with modified pits (Fig. 29).....20
More than one sclerite in presternum of prothorax (*prst*, Fig. 27); nasale of one single-pointed tooth; without conspicuous punctures on dorsum of abdominal segments (although sometimes with many fine, pin-point punctures).....21

20. Widely distributed; mainly in well-watered soils. Eyes absent.....
.....*Hemicrepidius memnonius* and related spp.
Normally inhabiting decaying wood and leaf litter. Eyes present.....**Athous* spp.

† *The characters used in this couplet unavoidably are rather minute and therefore difficult. The critical feature here is the presence or absence of the setae ped and atm as shown in Fig. 25; the additional characters enumerated are merely supplementary. In a contracted larva, the setae atm may be covered by the intersegmental membranes. It should also be noted (as revealed in Couplet 18) that Cryptohypnus abbreviatus is the only species of economic importance that falls in the first half of Couplet 17. If the specimen under examination does not agree with Figs. 25, 48, and 50 or with the notes given in the discussion of the genus Cryptohypnus (p. 369) then the species falls in the second half of the couplet or is not known to be a pest species.*

21. Soil inhabiting. Tips of outer urogomphal prongs curving backward when not eroded (*opr*, Figs. 51, 52, 53); several spine-like setae on episterna of meso- and metathorax (Fig. 28) 22
Normally inhabiting decaying wood and leaf litter. Tips of outer urogomphal prongs either curving forward or bluntly rounded; without spine-like setae on episterna of meso- and metathorax ..... **Ludius* spp.

22. Urogomphal prongs like grappling hooks, dark and horny (Fig. 51); four or more fine setae on central dorsal area of ninth abdominal segment (Fig. 55). Saskatchewan and Alberta ..... *Ludius sexualis* (?)
Urogomphal prongs less curved, fleshier (Figs. 52, 53); none or four setae on central dorsal area of ninth abdominal segment 23

23. Four setae on central dorsal area of ninth abdominal segment (Fig. 56). Okanagan Valley, B.C.; in semiarid areas ..... *Ludius pruininus*
Without setae on this area 24

24. With sharp, horny protuberances on lateral margins of dorsum of ninth abdominal segment (*to*, Fig. 54); urogomphi relatively slender (Fig. 52; *ur*, Fig. 54) 25
With rounded protuberances on these margins (*to*, Fig. 57); urogomphi short and thick (Fig. 53; *ur*, Fig. 57) ..... *Ludius aeripennis*
Confined to the grasslands and adjacent park areas of the Prairie Provinces, including much of the Peace River section of Alberta and British Columbia; rarely exceeding 22 mm. in length when mature ..... subsp. *destructor*
Inhabiting the mountainous and forested regions to the west and north of the prairie grasslands, including the coastal area of British Columbia; usually exceeding 25 mm. in length when mature ..... subsp. *aeripennis*

25. Okanagan Valley, B.C., and southern Alberta ..... *Ludius glaucus*
Eastern Canada ..... **Ludius inflatus* (?)

26 (15). With two conical protuberances anteriorly on dorsum of ninth abdominal segment (*pro*, Fig. 64); outer prongs of urogomphi very small (*opr*, Fig. 64). Widely distributed through northern parklands ..... *Limonius pectoralis*
Without such protuberances 27

27. Extremely small outer prongs on urogomphi (*opr*, Fig. 58) 28
Prominent outer (upper) prongs on urogomphi (*opr*, Figs. 59, 60, 61, 62, 63) 29

28. In dry situations. Larvae known only from dry-farmed fields at Lethbridge, Alta. ..... Unidentified, probably *Limonius* sp. (Group 1)
In moist situations: muck soils or leaf litter. Widely distributed ..... *Limonius aeger*

29. More than one sclerite comprising presternum of prothorax (*prst*, Fig. 27); nasale of one single-pointed tooth (Fig. 16); eyes present; inner prongs of urogomphi flattened, outer prongs horn-shaped (Fig. 63). Widely distributed in parklands soil ... *Ludius kendalli*
One large triangular sclerite comprising presternum of prothorax (*prst*, Fig. 24); nasale of one triple-pointed tooth (lateral denticles sometimes worn off, see Couplet 31) 30

30. Soil inhabiting. Eyes absent 31
Normally inhabiting decaying wood and leaf litter. Eyes present ..... **Ludius* spp., **Athous* spp., and some related non-economic genera

31. With prominent tubercle-like protuberances along lateral margins of dorsum of ninth abdominal segment, sharply pointed when not eroded (*to*, Figs. 59, 60); lateral denticles of tip of nasale small (Fig. 17), frequently eroded giving appearance of single-pointed tooth 32
With comparatively inconspicuous wavy expansions in this location (*to*, Fig. 62); denticles of nasale subequal in size, rarely eroded completely 33

32. With five or more tooth-like protuberances along lateral margins of dorsum of ninth abdominal segment (*to*, Fig. 60); larvae usually less than 20 mm. in length. In parklands west of Great Lakes ..... *Ludius limoniiformis* (?)
With four protuberances in position described above (Fig. 59); larvae may exceed 30 mm. in length. Eastern Canada ..... *Ludius cylindriformis* (?)

33. British Columbia ..... *Limonius canus*
Eastern Canada (Figs. 26, 61, 62) ..... *Limonius ectypus* (?)
Prairie Provinces (Fig. 7) ..... *Limonius* sp. (Group 2)

Discussion by Taxonomic Groups

Elaterid larvae generally can be separated easily into one or other of the four subfamilies, Cardiophorinae, Oestodinae, Elaterinae, and Pyrophorinae, as recognized by Böving and Craighead (3).

Subfamily Cardiophorinae ("false-segmented" wireworms)

The *Cardiophorus* type (Fig. 1) is very different from other wireworms, having a thread-like body with nodulated pseudo-segmentation, forked mandibles, and two digitate anal processes. Such larvae are distributed widely over Canada, occurring in very sandy areas and to a lesser extent in light loam soils, and are probably chiefly predatory and non-economic. Larvae identified as *Cardiophorus* sp. have been reported (13, p. 28) as being destructive to corn in Illinois, but there are no Canadian records of their attack on plants. The *Cardiophorus* larva closely resembles that of *Horistonotus uhlerii* Horn, a species of considerable economic importance (1, 28, 55) in southeastern United States.

Subfamily Oestodinae

The *Oestodes* type (Fig. 5) is rare, but specimens have been recorded from Alberta to the Atlantic Coast. *Oestodes puncticollis* has been known to damage spring wheat and oats in alkaline parts of fields near Wadena, Sask., and to a lesser degree at Cadogan, Alta. *O. tenuicollis* occurs in Manitoba and Eastern Canada, but has not been associated with plant injury. Larvae of *tenuicollis* have not been reared, but the distribution of the species, based on adult collections, leaves little doubt as to the identity of the *Oestodes* larvae found in the East. The large dorsal prongs (*dpr*) on the ninth abdominal segment, and the greatly reduced nasale (sometimes absent) are characteristic of this genus. Further structural details are given by Hyslop (30, p. 251) and Böving and Craighead (3, Pl. 83).

Subfamily Elaterinae ("pointed-tail" or "round-tail" wireworms)

Larvae of the Elaterinae (Figs. 2, 3, 4) are characterized mainly by the posterior end which may be round or flat, blunt or sharp, but never notched in the middle. The body is cylindrical with greatly reduced pleural membranes. Generally speaking, the larvae thrive in moist or wet situations and possibly have a tendency to feed on decaying rather than on living vegetable matter, as observed by Ghilarov (14, pp. 634–635) for certain European species. In spite of this tendency several species become sufficiently abundant under Canadian farming and gardening practices to cause severe damage to cultivated crops. Many species are wood-inhabiting and of no economic importance; among these are all known larvae of *Ampedus* Dej. (= *Elater* auct.) and *Parallelostethus* O. Schw. and many species in other genera. The Canadian species of *Agriotella* Brown (5) are not known in the larval stage.

Genus *Agriotes* Eschscholtz

(Figs. 3, 22, 23, 33, 34, 39, 40)

Three Canadian species are known in the larval stage, *mancus*, *criddlei*, and *limosus*. In all of these the ninth abdominal segment (Figs. 33, 34, 40) terminates in a blunt point which distinguishes *Agriotes* from the closely related genus *Dalopius*.

A. mancus (Fig. 3) is pale yellow and frequently exceeds 20 mm. in length when full grown. It is readily distinguished by the two pigmented "eye spots" near the front of the ninth abdominal segment (*es*, Fig. 33), a character that is entirely lacking in *criddlei* and *limosus* (Fig. 34). *A. mancus* occurs in Manitoba and is widely distributed through Eastern Canada where it occurs mainly in poorly drained land, especially in muck soils and old meadows, and in cultivated fields where grasses have been grown in recent years. Moisture appears to be the principal factor governing its distribution since it has been taken in abundance from fields of very different soil types. Forbes (13, p. 34) states that the larva of *A. pubescens* Mels. is inseparable from that of *mancus*. Since adults of *pubescens* have been collected in Manitoba and in other parts of Canada, it is possible that in some sections the larvae of these species might be confused. However, all larvae of this type that have been reared to date at the Saskatoon Laboratory have proved to be *mancus*. These rearings include material from Manitoba, Ontario, Quebec, and New Brunswick. Much has been written on the ravages and control of this pest (22, 24, 25, 26, 45, 46, 47).

A. criddlei is reported (58) to be the smallest American species of *Agriotes*. The pale brown larva rarely exceeds 10 mm. in length. It is the only known *Agriotes* larva that possesses preapical setiferous tubercles on the ninth abdominal segment (Fig. 40). In this respect it resembles *Dalopius* larvae, but the latter have more prominent tubercles and a sharper point on the ninth abdominal segment. Larval specimens have been taken in southwestern Saskatchewan and in southern Alberta, including the irrigated districts. However, the species is known to occur as far east as Aweme, Man., as far west as Chilcotin, B.C., and as far north as Saskatoon, Sask. It is a larva of this type that is referred to by Strickland (51, p. 4) as ". . . . a small wireworm, which appears to be the larva of some species of *Agriotes*", and again by King (34, p. 705) as "*Agriotini*? . . . a rather small species of considerable importance in southwest Saskatchewan."

A. limosus is a pale brown larva measuring 18 or 19 mm. when full grown. It lacks both the "eye spots" of *mancus* and the "tubercles" of *criddlei*. It is a relatively rare species, but is widely distributed in the soil of park and forest areas and is believed to be of some economic importance in northern Saskatchewan. The larva has been described in detail (18).

An unidentified larva, which may belong to this genus, was collected by R. Glendenning of the Dominion Entomological Laboratory, Agassiz, B.C., on decaying turnips near the forest's edge of Pemberton, B.C. This larva resembles *A. limosus*, but has the tip of the ninth abdominal segment produced into a short, nipple-like point (Fig. 39).

Genus *Dalopius* Eschscholtz

(Figs. 2, 19, 36, 37)

The larvae of three species of *Dalopius*—*mirabilis*, *pallidus*, and *parvulus*—have been reared from cultivated fields. In all of these the ninth abdominal segment (Figs. 36, 37) terminates in a *sharp* point and bears three whorls (sometimes only two whorls in small specimens) of preapical setiferous tubercles. All are pale brown larvae rarely exceeding 15 mm. in length. Park and forest regions appear to be the typical habitat; the only specimens taken in open prairie districts have been collected from river flats and moist ravines. Specific larval characters have not been worked out.

D. pallidus occurs from Alberta to New Brunswick. It has been reported as a crop pest at Wetaskewin, Alta., and in the muck soils of Ontario and Quebec. In Saskatchewan, larvae have been taken from leaf litter on the banks of the South Saskatchewan River at Saskatoon. *D. parvulus* (Fig. 2) appears to be associated with grassy situations in the more open park sections of Saskatchewan and Manitoba, causing injury to grain crops when such land is put under cultivation. *D. mirabilis* is known (6, p. 95) from Alberta to Quebec and larvae have been taken from cultivated fields at a number of points in northern and eastern Saskatchewan and at Findlay, Man. Larvae were abundant under horse and cow manure in a native grass pasture at Mozart, Sask., in a definitely alkaline situation. Ten species of *Dalopius* are recorded (6, p. 35) from British Columbia, but the larvae are unknown.

Genus *Melanotus* Eschscholtz

(Figs. 4, 30, 31)

While specific characters are poorly known for *Melanotus* larvae, the genus is readily recognized by the ninth abdominal segment (Figs. 30, 31) which is flattened at the tip and usually more or less scalloped or lobed posteriorly. Excellent supplementary characters are enumerated in the key, Couplet 3. The larvae (Fig. 4) are somewhat darker than most wireworms, being brown or reddish brown with the head, thorax, and ninth abdominal segment slightly darker, the venter slightly paler. The more common species measure 20 to 30 mm., or more, when full grown. Larvae of this genus occur in abundance throughout Eastern Canada, and to some extent in the heavy clay soil of the Red River Valley in Manitoba and along some of the river courses as far west as the Saskatchewan–Manitoba boundary. The available records, while not extensive, suggest that in Canada the chief habitat is poorly drained land or soils with good moisture-holding capacity. Light infestations have been reported in muck soils. This is in general accord with most observations (11, 12, 27) on American species. However, in the State of Maine, *Melanotus* larvae are reported (24, p. 8; 26, p. 55) to be more numerous in light, relatively dry soil. On the basis of characters given by Forbes (13) the most abundant economic species in Canada appear to belong to the *communis* Gyll.—*fissilis*

Say group. In economic importance, *Melanotus* larvae rank second to *Agriotes mancus* throughout Eastern Canada. Damage has been reported (41, p. 530; 44, p. 3) to be worse after the ploughing of sod.

SUBFAMILY PYROPHORINAE ("forked-tail" or "notched-tail" wireworms)

The larvae of this subfamily (Figs. 6 to 11) are readily distinguished from those of the Elaterinae by the presence of a median notch at the posterior extremity. The body is more or less dorsoventrally flattened, usually with well-developed lateral membranes (*mem*, Fig. 26). Food and moisture requirements are diverse and some groups are highly predacious. At present, only six genera are known to be of economic importance in Canada, the larvae of most other genera apparently being confined to decaying wood and leaf mould. For the most part, the genera of this group are not as easily separated by larval characters as in the Elaterinae, combinations of characters being necessary for generic identification.

Genus *Aeolus* Eschscholtz (=*Drasterius* auct.)

(Figs. 6, 15, 20, 44, 47)

Only one species, *Aeolus mellillus*, is known in Canada. In the economic literature it is commonly referred to as *dorsalis* Say or *elegans* auct. Three subspecies have been recognized (4), but these are not separable in the larval stage. The larva (Fig. 6) is distinguished readily among the wireworm pests in Canada by the brown or reddish-brown head and prothorax which contrasts vividly with the pale yellow or whitish abdomen. The body is decidedly flat and specimens rarely exceed 15 mm. in length. The larva is notoriously predacious and its activity is noticeably greater than that of most wireworms. Damage by this wireworm frequently resembles the work of cutworms, the point of attack usually being near the soil surface and, in the case of young grain plants, the stems often are completely cut off. By contrast other wireworms bore into and shred the stems, but rarely sever them completely. Other diagnostic characters are given in the key, Couplet 14, and fairly detailed descriptions of the larva are given by Comstock and Slingerland (10) and by Jewett (31). Apparently the life cycle is completed normally in one year and development is by parthenogenesis in Canada and in some sections of the United States (33, 50). The species is found throughout Western Canada where it is a minor pest of cereals (34), particularly in southern districts and occurs as far east as Ontario where it has been reported (50) as severely injuring tobacco and truck crops in the southwestern peninsula. It inhabits native grassland and may be noticed as soon as such land is ploughed, but it also occurs commonly in old cultivated fields. Relatively dry situations seem to be preferred, although infestations occur in irrigated land and in soils of a wide range of texture. There are numerous records of plant injury by this pest in the United States (10, 13, 27, 31, 32, 49, 59). *Aeolus* is closely related to *Conoderus* Esch. (=*Monocrepidius* Esch.) and *Heteroderes* Latr., genera of economic significance in the United States.

Genus *Cryptohypnus* Eschscholtz

(Figs. 8, 25, 45, 46, 48, 50)

Accurate identification of the larvae of *Cryptohypnus* depends upon a combination of rather obscure characters. However, among the known wireworm pests of Canada, *Cryptohypnus* alone possesses the setae, labelled *ped* and *atm* in Figs. 8 and 25. Only two species, *nocturnus* and *abbreviatus*, are of economic significance in the Dominion. Both of these are pale yellow or bright yellow larvae with a large caudal notch, and reach 10 to 12 mm. in length when mature.

C. nocturnus (Fig. 8) is readily distinguished by the small, erect outer urogomphal prongs (*opr*, Figs. 45, 46) and by the U-shaped caudal notch which is not narrowed posteriorly. The larva has been described in detail (2). Adults of *nocturnus* have been taken from coast to coast in Canada, but records of crop injury are confined to Manitoba, Saskatchewan, Alberta, and the Peace River area of British Columbia. Over this range it ranks second to *Ludius aeripennis* as a pest of grain crops and surpasses it in importance in parts of the irrigated areas of Alberta. Although distributed throughout the open prairie and parklands it is relatively more abundant in areas of heavy soil, in grassy situations, and in fields that recently have been broken from sod (34). Control recommendations are discussed by King *et al.* (35, 36, 37).

In *C. abbreviatus* the urogomphal prongs (Figs. 48, 50) are subequal in length and the caudal notch is considerably narrowed posteriorly by the incurving tips of the inner prongs. A detailed description of the larva is given by Comstock and Slingerland (10, p. 270). This species is distributed throughout Eastern Canada, the most westerly record being Rhein, Sask. It inhabits soils of various textures, but appears to be more abundant in poorly drained land. In muck soils it is a minor pest.

Genus *Hemicrepidius* Germar (=*Asaphes* Kirby)

(Figs. 10, 29, 49)

Hemicrepidius larvae (Fig. 10) are bright yellowish-brown, and 20 to 30 mm. in length when mature. The large caudal notch (*cn*, Fig. 49), conspicuous sculpturing on the anterior half of the abdominal segments (Fig. 29), and the lack of eyes, when taken together distinguish the genus. Specific characters have not been worked out, but the larva of *H. hemipodus* Say (= *H. decoloratus* Say) has been described (10, 13). *H. memnonius* is the only Canadian pest species for which the larva has been identified by rearing. Although distributed throughout the Dominion, the larvae are rarely encountered in Western Canada and even in the East have never been reported as abundant.. Moist situations such as watered gardens, muck soils, and low-lying land appear to be the favoured habitat.

Genus *Hypnoidus* Stephens

(Figs. 11, 21, 43)

Only one species, *Hypnoidus dubius*, has been encountered in cultivated fields in Canada. It is readily distinguished from other wireworm pests by the sharp, undivided incurving urogomphi (Fig. 43) and the large caudal notch. The larva (Fig. 11) rarely exceeds 10 mm. in length, has a soft, flattened body and is very pale yellow with slightly darker head and prothorax. It inhabits sandy loam soils in the open prairie sections of Saskatchewan, but is not definitely known to injure crops.

Genus *Limonius* Eschscholtz (=*Pheletes* Kiesenwetter)

(Figs. 7, 18, 24, 26, 58, 61, 62, 64)

Limonius larvae are bright yellow or yellowish-brown. The pest species can be distinguished from other Canadian wireworm pests by the following combination of characters: nasale of one triple-pointed tooth (as in Figs. 18, 19), small caudal notch (*cn*, Figs. 58, 62, 64), and well-rounded comparatively inconspicuous protuberances along the lateral margins of the dorsum of the ninth abdominal segment (Figs. 58, 62, 64). Although widely distributed in Canada the species are usually found in moist, and sometimes, alkaline situations. Similar observations on this genus are recorded by Lane (42, p. 530).

The larvae of the Canadian species of *Limonius* are poorly known; only *pectoralis*, *aeger*, *canus*, a species "near *ectypus* Say", and *consimilis* Walker (non-economic) have been identified through rearing. Although *Limonius* larvae can be recognized by the characters listed above, it facilitates identification of the pest species if these be considered as two distinct groups.

Group 1 includes *pectoralis*, *aeger*, and an unidentified larva. These species belong to different natural groups (17), but for convenience are considered here as a single unit. The larvae all have the outer urogomphal prongs reduced to mere tubercles (*opr*, Figs. 58, 64) and thus are distinguished from other wireworms having a small caudal notch. *L. pectoralis* measures up to 14 mm. in length and is readily distinguished by the two conical protuberances on the anterior part of the dorsum of the ninth abdominal segment (Fig. 64) and by the nasale in which the middle denticle is smallest (Fig. 18). It occurs widely in the Dominion, but only in the deep, black soils of the park belt of the Prairie Provinces and in the Peace River section of British Columbia is it known to be an important crop pest. In virgin land its typical habitat seems to be the grassy margins of snowberry (*Symphoricarpos*) thickets (34). Larvae believed to be *L. aeger* occur in the muck soils of southern Quebec where they are injurious to truck crops. However, in the Prairie Provinces the species has never been found in cultivated land although the larvae have been collected, and on several occasions reared to maturity, from moist leaf litter on the banks of the Saskatchewan River at Saskatoon. The unidentified species (Fig. 58) is known only from Lethbridge, Alta.,

and was taken by G. F. Manson of the Lethbridge Laboratory from a dry-farmed corn field where it caused considerable damage. Adults of *Limonius ursinus* Van Dyke have been collected in this locality.

Group 2 includes *canus*, *ectypus* (?), and a third species of uncertain identity. All are relatively large species, sometimes attaining 25 mm. in length. These larvae differ from *Group* 1 by having the outer urogomphal prongs about as long as the inner prongs (Figs. 61, 62) and may be distinguished from other pest species that have a small caudal notch by the combination of a triple-pointed nasale and relatively inconspicuous, well rounded protuberances along the lateral margins of the ninth abdominal segment (*to*, Fig. 62). Structurally, these species are very similar.

Canus and *ectypus* are almost indistinguishable. Both have long tergal impressions (*im*, Fig. 26) which, although varying considerably in length, nearly always clearly fall short of the mid-dorsal line. *L. canus* is a well-known pest in irrigated land and has been reported (48) as injuring truck and grain crops in the interior and coastal regions of British Columbia. The larva has been described in detail by Lanchester (38). *L. ectypus* (Figs. 26, 61, 62) is an eastern species and is known to attack tobacco and truck crops in southwestern Ontario.

The third species, while closely related to *canus* and *ectypus*, is distinct in both adult and larval stages. One adult has been reared, but specific identification has not yet proved possible. The larva is characterized by having tergal impressions that usually extend to the mid-dorsal line on the second to seventh abdominal segments (*im*, Fig. 7). Damage by this wireworm has been observed to cereals growing in low-lying, alkaline areas in open prairie and parkland margins in Manitoba, Saskatchewan, and Alberta. Usually only small spots in a field are affected. A few specimens have been found in irrigated land in Alberta.

Further information on the life-history, economic importance, and control of *Limonius* larvae is given by Graf (23) and by Lane (39, 41, 42, 43).

Genus *Ludius* Eschscholtz (= *Corymbites* Latreille)

(Figs. 9, 12, 13, 16, 17, 27, 28, 51 to 57, 59, 60, 63)

The larvae of seven species of *Ludius* have been taken from cultivated fields in Canada. These are too diverse in structure to be described as a single unit, and for practical purposes* are considered here as three groups.

Group 1 includes *sexualis*, *glaucus*, *inflatus*, *pruininus*, and *aeripennis*. These bright yellow larvae have a large caudal notch (*cn*, Figs. 54 to 57), the presternum of the prothorax divided into two or more sclerites (*prst*, Fig. 27), and lack the small setae that characterize *Cryptohypnus* (*ped*, *atm*, Figs. 8, 25). Specific separation depends entirely upon characters of the ninth abdominal segment.

* *In a study (17) of the known Ludius larvae of the world, 20 distinct "species groups" have been recognized. For convenience, Group 1 in this paper includes the economic species from several distinct, but closely related, Canadian groups.*

In *L. sexualis* (Fig. 55) this segment is almost square, with inconspicuous lateral protuberances (*to*), with four or more small hairs on the central dorsal area, and with the urogomphal prongs (Fig. 51) dark, horny, and converging much like grappling hooks. Specimens seldom exceed 16 mm. in length. Larvae have not been reared, but the identification is believed to be correct. The species is known from Alberta and Saskatchewan where it is associated in native situations with prairie grasses, especially in mixed grass and silverberry (*Elaeagnus*), and also to some extent in the grassy margins of snowberry (*Symphoricarpos*) thickets. It persists for many years after such areas are put under cultivation.

L. glaucus and *L. inflatus* are almost indistinguishable structurally and must be identified on the basis of distribution. These have relatively slender urogomphi (Figs. 52, 54), prominent tooth-like lateral projections (*to*), and no setae on the central dorsal area of the ninth abdominal segment. The largest specimens examined measured 18.5 mm. in length. Adults of *glaucus* have been taken (8) from the Okanagan Valley, B.C., and from southern Alberta. Larvae are known to injure grain crops in southwestern Alberta and along the foothills as far north as Calgary. Under the name "*inflatus* Say", damage by larvae of *glaucus* is reported by Hyslop (27, 29) and by Lane (39) in the northwestern United States. The true *inflatus* inhabits Eastern North America (8) and as far as is known is of no economic significance.

L. pruininus Horn (= *L. noxius* Hyslop), the great basin wireworm, is characterized (Fig. 56) by the stout urogomphi and relatively fleshy urogomphal prongs, the rather prominent lateral protuberances (*to*), and the four setae on the central dorsal area of the ninth abdominal segment. The larvae may exceed 20 mm. in length. In Canada, this species has been reported from the Okanagan Valley, B.C. It is a well known dry-land pest in the northwestern United States (27, 39, 40, 42), where its distribution is limited to the area that normally receives not more than 15 in. of rainfall annually.

L. aeripennis (Figs. 53, 57) closely resembles *pruininus*, but has less prominent protuberances (*to*) and lacks setae on the central dorsal region of the ninth abdominal segment. The typical *aeripennis* may attain 28 mm. in length, but the subspecies *destructor* rarely exceeds 22 mm. *L. aeripennis destructor* (Fig. 9), the prairie grain wireworm, is the outstanding wireworm pest in Canada. It is present over the whole agricultural section of the West, including the Peace River Block of Alberta and British Columbia; and also in the northern Great Plains states. Being a typical dry-land subspecies it is more abundant and more injurious on the well drained light and medium soils of the open prairies, but is also a pest in irrigated sandy soil. Grains and grasses are its favourite food and fields planted to these for five or more years in succession, without an intervening year of summer-fallow, usually become severely infested. The morphology of the larva has been described (15, 16, 17), its economic importance, ecology, and biology discussed (34, 52, 53, 54), and control measures enumerated (19, 20, 35, 36, 37, 52).

In the forested and mountainous areas to the north and west of the grasslands, the subspecies *destructor* is replaced by the larger typical *aeripennis* (7), which is a pest of truck crops and cereals in some of the mountain valleys and on the coastal area of British Columbia. Both subspecies are present in at least part of the Peace River Block of Alberta and British Columbia.

Group 2 is represented in Canada by the single species *L. kendalli* (= *L. virens* of American authors). This yellow-brown larva may attain 28 mm. in length and differs from those in *Group* 1 by having a small caudal notch (*cn*, Fig. 63). The divided prothoracic presternum (*prst*, Fig. 27) and the single-pointed nasale (Fig. 16) separate it from *Limonius* larvae. The species is widely distributed over the Dominion in northern parkland and forest (9), but records of damage are limited to northern Saskatchewan where, in localized areas, it is a serious pest for three or four years following the breaking of willow scrub land.

Group 3 includes *L. cylindriformis* and *L. limoniiformis*. The larvae of these species are yellow or yellowish-brown and have a small caudal notch (*cn*, Figs. 59, 60), the presternum of the prothorax undivided (*prst*, Fig. 24), and lack eyes. In these characters they resemble the pest species of *Limonius* from which they are distinguished by the small but prominent tubercle-like protuberances along the lateral margins of the dorsum of the ninth abdominal segment (*to*, Figs. 59, 60). Both species seem to prefer relatively moist situations. The only larvae of *cylindriformis* available to the authors were taken from a garden at Springhill, N.B., and were received through the courtesy of the Fredericton Laboratory. Adults are recorded from Nova Scotia and throughout southern Quebec and Ontario. The species is reported (26) to be of some economic significance in the State of Maine. Larvae believed to be *L. limoniiformis* have been taken from cultivated fields in the parklands of Saskatchewan; the adults are known (58, pp. 396, 420) from a wide area west of the Great Lakes, extending into Alberta.

Summary of the Regional Distribution of Pest Species

In Eastern Canada, which for present purposes includes a part of eastern Manitoba, the chief wireworm pests belong to the subfamily Elaterinae. The genera *Agriotes*, *Melanotus*, and *Dalopius* are widely distributed over this region, *A. mancus* being the most important pest species. In local sections of the East, severe losses are caused by wireworms of the subfamily Pyrophorinae, as in southwestern Ontario where *Aeolus mellillus* and *Limonius ectypus* frequently are troublesome. *Ludius cylindriformis*, *Cryptohypnus abbreviatus*, *Hemicrepidius memnonius*, and *Limonius aeger* have been reported as injurious in scattered localities.

In the muck soils—which are being increasingly used for vegetable production—the wireworms most commonly encountered are *Agriotes mancus*, *Dalopius pallidus*, and *Limonius aeger*. Species occurring in smaller numbers include *Cryptohypnus abbreviatus*, *Melanotus* spp., and *Hemicrepidius memnonius*.

The transition between the typically eastern and the typically western pest species is found in southeastern Manitoba. In the Red River Valley wireworms are not a serious pest, but in this area and further east *Agriotes mancus* and *Melanotus* spp. occur, sometimes together with a few specimens of *Ludius aeripennis* and *Cryptohypnus nocturnus*.

In Western Canada, the chief economic species are of the subfamily Pyrophorinae, mainly of the genera *Ludius*, *Cryptohypnus*, and *Limonius*. Larvae of *Dalopius* spp. and *Agriotes criddlei* are the only representatives of the subfamily Elaterinae that rank as important crop pests in this part of the Dominion while damage by larvae of the subfamily Oestodinae is extremely rare. *Ludius* larvae are found throughout this region. *Ludius aeripennis*, especially its subspecies *destructor*, is of outstanding economic importance.

In the open prairie country of Alberta, Saskatchewan, and Manitoba, and in the Peace River area of Alberta and British Columbia, *L. aeripennis destructor* predominates with *Cryptohypnus nocturnus* second in importance. *Limonius* sp. (near *ectypus*), *Hypnoidus dubius*, and *Aeolus mellillus* inhabit the southern prairie areas, but usually are present in smaller numbers and only in local situations. *Agriotes criddlei* is the only member of the Elaterinae inhabiting cultivated fields in typical open prairie locations. In the irrigated sections of southern Alberta, *C. nocturnus* is the chief pest of truck crops. Severe infestations of *L. destructor* are common in irrigated sandy land, but infrequent in the heavier soils, especially in fields that have received three or more floodings annually for several years. Larvae of *Aeolus mellillus*, *Agriotes criddlei*, and *Limonius* spp. occur in small numbers over the irrigated sections.

In the parklands of the Prairie Provinces, *L. aeripennis* and *C. nocturnus* are rather widely distributed, but the following species also may be present and sometimes predominant: *Limonius pectoralis*, *Ludius kendalli*, *Dalopius* spp., *Ludius sexualis*, *Ludius limoniiformis*, and *Agriotes limosus*. Rarely, in well watered sites, a few specimens may be encountered of *Hemicrepidius memnonius* and *Cryptohypnus abbreviatus*, but these have never been taken in abundance in the West.

The wireworms of British Columbia have not been studied carefully. *Limonius canus*, *Ludius aeripennis*, *Ludius glaucus*, and *Ludius pruininus* are pests in the Okanagan Valley and probably in other parts of the interior. *Aeripennis* and *canus* also occur along the Pacific Coast where they commonly injure truck crops. *Canus* is found chiefly in irrigated land while the others are typical dry land species.

List of Pest Species and Illustrations

The list is alphabetical, first by genera, then by species in each genus. Under "Distribution" is shown the region where each pest species is known to be, or likely to be, of economic importance. Page numbers refer to the principal discussions in the text. All identifications are believed to be correct,

but where there has not been verification by actual rearing a question mark is inserted. Certain non-economic species, included in the key as an aid to identification, are marked with an asterisk.

Species	Distribution	Figures	Page
Aeolus mellillus Say	S.W. Ontario, Prairie Provinces; probably British Columbia	6, 15, 20, 44, 47	368
Agriotes			365
criddlei Van Dyke	S.W. Saskatchewan, S. Alberta; probably throughout Western Canada	40	365
limosus Le Conte	Saskatchewan; probably Manitoba and Alberta	22, 34	365
mancus Say	Eastern Canada; probably eastern Manitoba	3, 23, 33	365
(?) sp.	British Columbia	39	366
**Ampedus* spp.		32	365
**Athous* spp.			
**Cardiophorus* spp.		1	365
Cryptohypnus			369
abbreviatus Say	Eastern Canada, Manitoba, eastern Saskatchewan	25, 48, 50	369
nocturnus Eschscholtz	Western Canada	8, 45, 46	369
Dalopius			367
mirabilis Brown	Manitoba, Saskatchewan; probably from Quebec to Alberta		367
pallidus Brown	Quebec, Ontario, Alberta; probably from New Brunswick to Alberta		367
parvulus Brown	Manitoba, Saskatchewan	2, 19, 36, 37	367
spp.	Throughout Canada		367
**Eanus decoratus* Mannerheim		42	
Eleodes spp. (false wireworms)		14, 35, 41	359
Hemicrepidius			369
memnonius Herbst	Throughout Canada, except British Columbia	47	369
spp.	Throughout Canada	10, 29	369
Hypnoidus dubius Horn	Saskatchewan, probably Manitoba and Alberta	11, 21, 43	370
Limonius			370
aeger Le Conte	Quebec; probably Eastern Canada		370
canus Le Conte	British Columbia		371
ectypus Say (?)	Ontario; probably Quebec	24, 26, 61, 62	371
pectoralis Le Conte	Western Canada	18, 64	370
sp. (Group 1) possibly *ursinus* Van Dyke	Southern Alberta	58	370
sp. (Group 2)	Prairie Provinces	7	371

Species	Distribution	Figures	Page
Ludius			371
aeripennis aeripennis Kirby	Western Canada (northern forests and western mountains and coast)		372
aeripennis destructor Brown	Prairie Provinces and Peace River Block, B.C. (open prairie and park)	9, 12, 13, 27, 28, 53, 57	372
cylindriformis Herbst (?)	New Brunswick; probably Eastern Canada	59	373
glaucus Germar	Southern Alberta, interior British Columbia	52, 54	371
**inflatus* Say (?)			371
kendalli Kirby	Saskatchewan, probably Prairie Provinces	16, 63	373
limoniiformis Horn (?)	Saskatchewan, probably Prairie Provinces	17, 60	373
pruininus Horn	Interior British Columbia	56	371
sexualis Brown (?)	Saskatchewan; probably Prairie Provinces	51, 55	371
*spp.			
Melanotus spp.	Eastern Canada, Manitoba	4, 30, 31	367
Oestodes			
puncticollis Horn	Saskatchewan, Alberta		365
tenuicollis Randall	Eastern Canada, Manitoba	5	365
**Parallelostethus attenuatus* Say		38	365

Acknowledgments

Grateful acknowledgment is made for the receipt of valuable material and notes, to E. H. Strickland, Professor of Entomology, University of Alberta, Edmonton, Alta., and to the officers of the various Dominion Entomological Laboratories, especially to our colleagues at Saskatoon; and for the identification of all reared adults to W. J. Brown, Systematic Entomology, Division of Entomology, Ottawa, Canada.

References

1. ANDERSON, G. M. The slender wireworm; its relations to soils. South Carolina Agr. Expt. Sta. Bull. 204. 1920.
2. ARNASON, A. P. A morphological study of the immature stages of *Cryptohypnus nocturnus* Eschscholtz and a study of some ecological factors concerning wireworms. Rept. to Can. Div. Entomol. and to Univ. Saskatchewan (M.Sc. Thesis). 1931.
3. BÖVING, A. G. and CRAIGHEAD, F. C. An illustrated synopsis of the principal larval forms of the order Coleoptera. Entomol. Am. 11 : 1-80, 81-160, 161-256, 1930; 257-351. 1931.
4. BROWN, W. J. Studies in the Elateridae. I. Can. Entomol. 65 : 133-141. 1933.
5. BROWN, W. J. Studies in the Elateridae. II. Can. Entomol. 65 : 173-182. 1933.
6. BROWN, W. J. The American species of *Dalopius* Esch. Can. Entomol. 66 : 30-39, 66-72, 87-96, 102-110. 1934.
7. BROWN, W. J. American species of *Ludius*; the *aeripennis* group. Can. Entomol. 67 : 125-135. 1935.

8. BROWN, W. J. American species of *Ludius*; the *inflatus* group. Can. Entomol. 68 : 133-136. 1936.

9. BROWN, W. J. Some American species of *Ludius* (Coleoptera). Can. Entomol. 71 : 44-49. 1939.

10. COMSTOCK, J. H. and SLINGERLAND, M. V. Wireworms. New York Cornell Agr. Expt. Sta. Entomol. Div. Bull. 33 : 193-272. 1891.

11. CONRADI, A. F. Report of the Entomology Division. Ann. Rept. South Carolina Agr. Expt. Sta. 31 : 27-33. 1918.

12. FENTON, F. A. Observations on the biology of *Melanotus communis* and *Melanotus pilosus*. J. Econ. Entomol. 19 : 502-504. 1926.

13. FORBES, S. A. Wireworms. *In* Eighteenth report of the State Entomologist on the noxious and beneficial insects of the State of Illinois for the year 1891-1892, 24-44. 1892. (Reprinted by authority of the State of Illinois, Springfield, Ill. 1920.)

14. GHILAROV, M. S. The fauna of injurious soil insects of arable land. Bull. Entomol. Research, 28 : 633-637. 1937.

15. GLEN, R. The external morphology and the characters separating the Elaterid larvae of Saskatchewan; a preliminary study. Rept. to Can. Div. Entomol. and to Univ. Saskatchewan (M.Sc. Thesis). 1931.

16. GLEN, R. Contributions to the morphology of the larval Elateridae (Coleoptera); No. 1, *Ludius aeripennis destructor* Brown. Can. Entomol. 67 : 231-238. 1935.

17. GLEN, R. Larval morphology and taxonomy of the tribe Lepturoidini with special reference to the genus *Ludius* Esch. (Coleop., Elateridae). Rept. to Can. Div. Entomol. and to Univ. Minnesota (Ph.D. Thesis). 1940.

18. GLEN R. Contributions to the morphology of the larval Elateridae (Coleoptera); No. 2, *Agriotes limosus* Leconte. Can. Entomol. 73 : 57-62. 1941.

19. GLEN, R. and KING, K. M. The relation of wireworms to potato growing in the Prairie Provinces. Sci. Agr. 18 : 283-287. 1938.

20. GLEN, R., KING, K. M., and ARNASON, A. P. The wireworm problem of garden, potato and truck crops in the Prairie Provinces. Can. Dept. Agr., Entomol. Branch, Saskatoon Leaflet No. 44. 1936.

21. GLEN, R., KING, K. M., and ARNASON, A. P. A field-man's key to the common wireworms of Canada. Proc. Am. Assoc. Adv. Sci., Rept. Entomol. Sect., p. 1. 1938.

22. GORHAM, R. P. Notes on *Agriotes mancus* Say at Dartmouth, N.S. Proc. Acadian Entomol. Soc. 1923, 9 : 69-72. 1924.

23. GRAF, J. E. A preliminary report of the sugar beet wireworm. U.S. Dept. Agr. Bur. Entomol. Bull. 123. 1914.

24. HAWKINS, J. H. Wireworms affecting Maine agriculture. Maine Agr. Expt. Sta. Bull. 343. 1928.

25. HAWKINS, J. H. Wireworm control in Maine. J. Econ. Entomol. 23 : 349-352. 1930.

26. HAWKINS, J. H. The bionomics and control of wireworms in Maine. Maine Agr. Expt. Sta. Bull. 381. 1936.

27. HYSLOP, J. A. Wireworms attacking cereal and forage crops. U.S. Dept. Agr. (Professional Paper) Bull. 156. 1915.

28. HYSLOP, J. A. Notes on the habits and anatomy of *Horistonotus uhlerii* Horn. Proc. Entomol. Soc. Wash. 17 : 179-185. 1915.

29. HYSLOP, J. A. Wireworms destructive to cereal and forage crops. U.S. Dept. Agr. Farmers' Bull. 725. 1916.

30. HYSLOP, J. A. The phylogeny of the Elateridae based on larval characters. Ann. Entomol. Soc. Am. 10 : 241-263. 1917.

31. JEWETT, H. H. Four wireworms found in bluegrass sod. Kentucky Agr. Expt. Sta. Bull. No. 392. 1939.

32. JEWETT, H. H. Wireworm injury to tobacco plants. Kentucky Agr. Expt. Sta. Bull. No. 398. 1940.

33. JEWETT, H. H. Observations on life history of *Aeolus mellillus*. J. Econ. Entomol. 33 : 816. 1940.

34. KING, K. M. Economic importance of wireworms and false wireworms in Saskatchewan. Sci. Agr. 8 : 693-706. 1928.

35. KING, K. M., ARNASON, A. P., and GLEN, R. The wireworm problem in field crops of Western Canada. Can. Dept. Agr., Entomol. Branch, Saskatoon Leaflet No. 35. 1933.

36. KING, K. M. and GLEN, R. The wireworm problem in field crops of Western Canada, a summary. Can. Dept. Agr., Entomol. Branch, Saskatoon Leaflet No. 43. 1936.

37. KING, K. M., GLEN, R., MCMAHON, H., and ARNASON, A. P. Wireworm control in western grain fields. Can. Dept. Agr., War-Time Prod. Ser. Spec. Pamphlet No. 37. 1940.

38. LANCHESTER, H. P. The external anatomy of the larva of the Pacific Coast wireworm. U.S. Dept. Agr. Tech. Bull. 693. 1939.

39. LANE, M. C. The economic wireworms of the Pacific Northwest (Elateridae). J. Econ. Entomol. 18 : 90-95. 1925.

40. LANE, M. C. The Great Basin wireworm in the Pacific Northwest. U.S. Dept. Agr. Farmers' Bull. 1657. 1931.

41. LANE, M. C. Control of wireworms on irrigated lands in the Pacific Northwest. U.S. Dept. Agr., Bur. Entomol. Plant Quarantine, Leaflet E-320. 1934.

42. LANE, M. C. Recent progress in the control of wireworms. Proc. World's Grain Exhib. Conf., 1933, 2 : 529-534. 1935.

43. LANE, M. C. Wireworms and their control on irrigated lands. U.S. Dept. Agr. Farmers' Bull. No. 1866. 1941.

44. MAIL, G. A. The wireworm. Univ. Minnesota, Agr. Ext. Div. Circ. 29. 1928.

45. PETTIT, J. Description of the wheat wireworm *Agriotes mancus* Say. Can. Entomol. 4 : 3-6. 1872.

46. RAWLINS, W. A. Experimental studies on the wheat wireworm, *Agriotes mancus* Say. J. Econ. Entomol. 27 : 308-314. 1934.

47. RAWLINS, W. A. Biology and control of the wheat wireworm, *Agriotes mancus* Say. New York Cornell Agr. Expt. Sta. Bull. 738. 1940.

48. RUHMANN, M. H. Report of the Entomology Branch. Ann. Rept. British Columbia Dept. Agr. 1930, 25 : G49-G51. 1931.

49. SLINGERLAND, M. V. Wireworms and the bud moth. New York Cornell Agr. Expt. Sta. Bull. 107 : 37-66. 1896.

50. STIRRETT, G. M. Notes on the "flat wireworm", *Aeolus mellillus* Say. Can. Entomol. 68 : 117-118. 1936.

51. STRICKLAND, E. H. Wireworms of Alberta. Univ. Alberta, Coll. Agr. Research Bull. No. 2. 1926.

52. STRICKLAND, E. H. Wireworms. *In* Insect pests of grain in Alberta. Univ. Alberta, Coll. Agr. Bull. 24 : 36-45. 1933.

53. STRICKLAND, E. H. The biology of prairie inhabiting wireworms. Proc. World's Grain Exhib. Conf., 1933, 2 : 520-529. 1935.

54. STRICKLAND, E. H. Life cycle and food requirements of the northern grain wireworm, *Ludius aeripennis destructor* Brown. J. Econ. Entomol. 32 : 322-329. 1939.

55. TENHET, J. N. and HOWE, E. W. The sand wireworm and its control in the South Carolina coastal plain. U.S. Dept. Agr. Tech. Bull. 659. 1939.

56. THOMAS, C. A. A review of research on the control of wireworms. Pennsylvania State Coll. Expt. Sta. Bull. 259. 1930.

57. THOMAS, C. A. The biology and control of wireworms; a review of the literature. Pennsylvania State Coll. Expt. Sta. Bull. 392. 1940.

58. VAN DYKE, E. C. Miscellaneous studies in the Elateridae and related families of Coleoptera. Proc. Calif. Acad. Sci. (Ser. 4) 20 : 291-465. 1932.

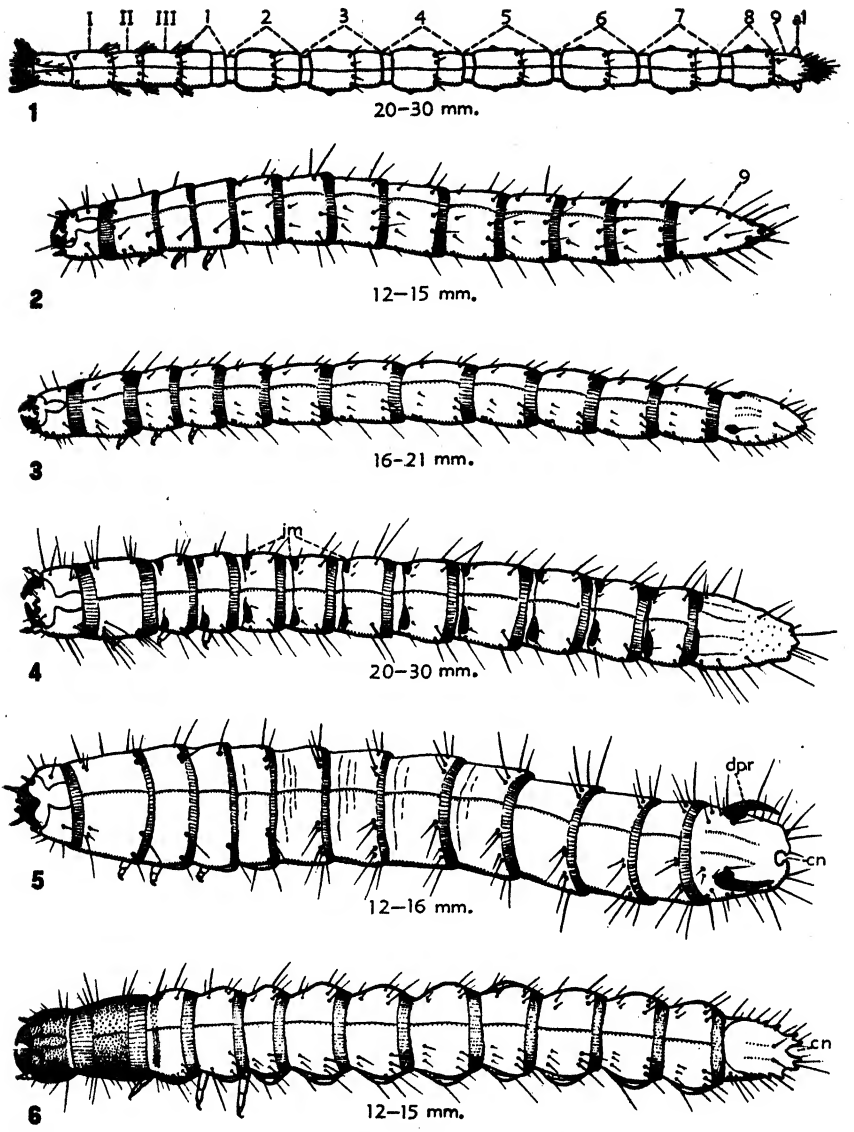
59. WEBSTER, F. M. Report of observations upon insects affecting grains. *In* U.S. Dept. Agr., Div. Entomol. Bull. 22. 1889.

Abbreviations Used in Illustrations

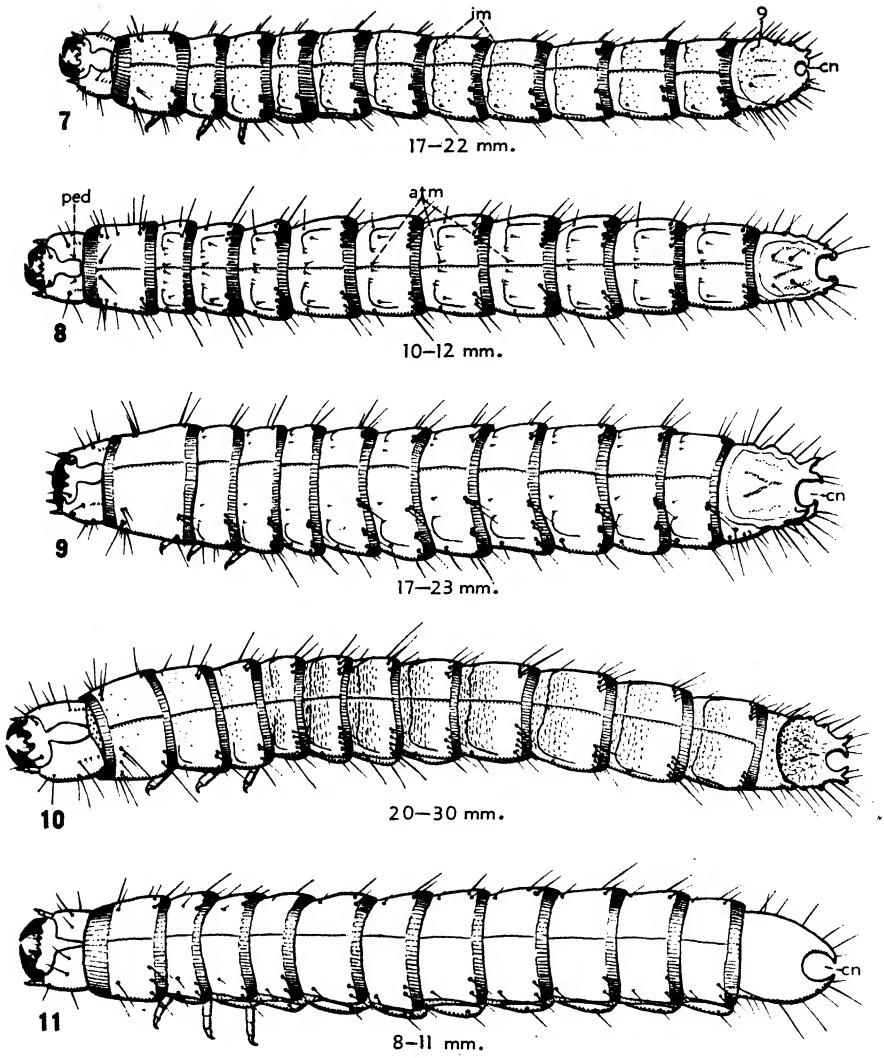
(See pp. 380-387)

Only the structures of primary taxonomic importance have been labelled. The terminology used was developed during recent detailed studies (17, 18).

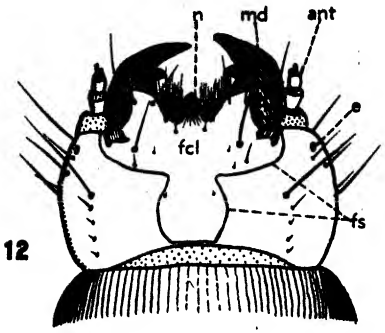
al, anal lobe
ant, antenna
ar, anal armature
atm, medial anterotergal seta
cl, clypeus (in "false" wireworms)
cn, caudal notch
cx, coxa
dpr, dorsal prongs
e, eye
epst, episternum
es, pigmented eye-like impressions or "eye spots" on ninth abdominal segment
fcl, frontoclypeal region
fr, frons (in "false" wireworms)
fs, frontal suture
im, impression
ipr, inner or lower prong of urogomphus
lbr, labrum (in "false" wireworms)
md, mandible
mem, membrane
n, nasale
opr, outer or upper prong of urogomphus
ped, dorsal posteroepicranial seta
pro, protuberance
prst, presternal area
ret, retinaculum
sp, spiracle
st, sternum
tg, tergum
to, tooth-like protuberance
tub, tubercle
ur, urogomphus (="cercus")
vmth, ventral mouth parts: the unit formed by the fusion of maxillae and labium
1 to 10, abdominal segments
I, *II*, *III*, thoracic segments



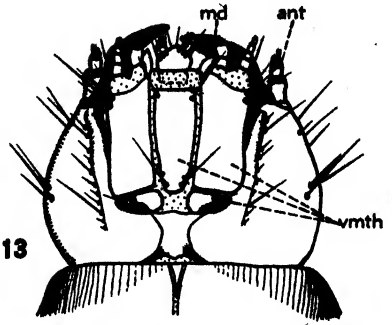
FIGS. 1 TO 6. *Types of wireworms (dorsal view). Length at maturity shown under each figure.* 1. *Cardiophorus sp.* 2. *Dalopius parvulus.* 3. *Agriotes mancus.* 4. *Melanotus sp.* 5. *Oestodes tenuicollis.* 6. *Aeolus mellillus.*



FIGS. 7 TO 11. *Types of wireworms (dorsal view). Length at maturity shown under each figure.* 7. *Limonius sp. (Group 2).* 8. *Cryptohypnus nocturnus.* 9. *Ludius aeripennis destructor.* 10. *Hemicrepidius sp.* 11. *Hypnoidus dubius.*

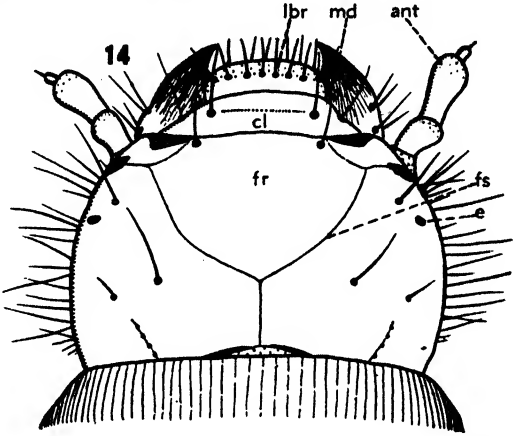


12



13

15



14

16

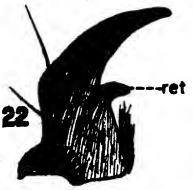
17

18

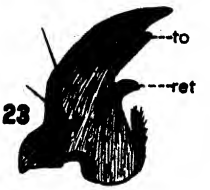
19

20

21

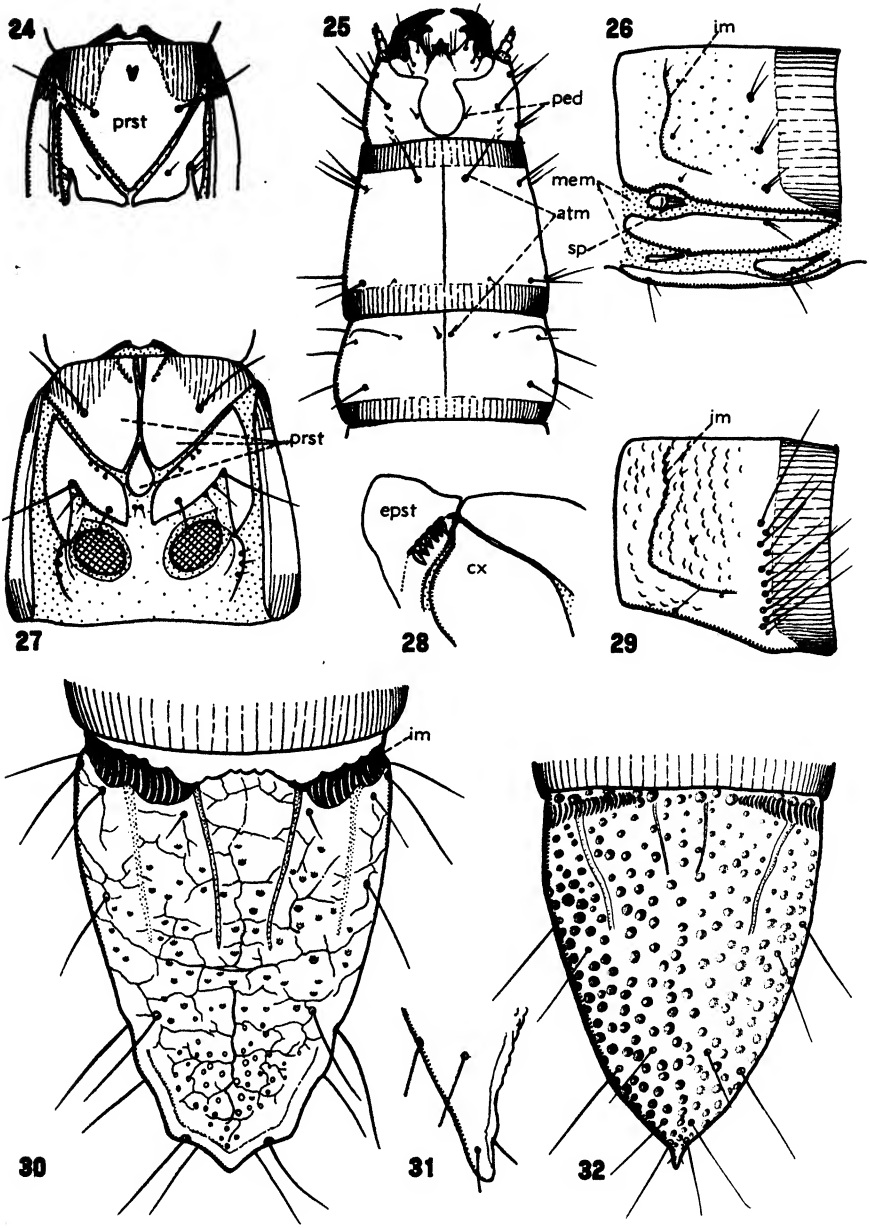


22

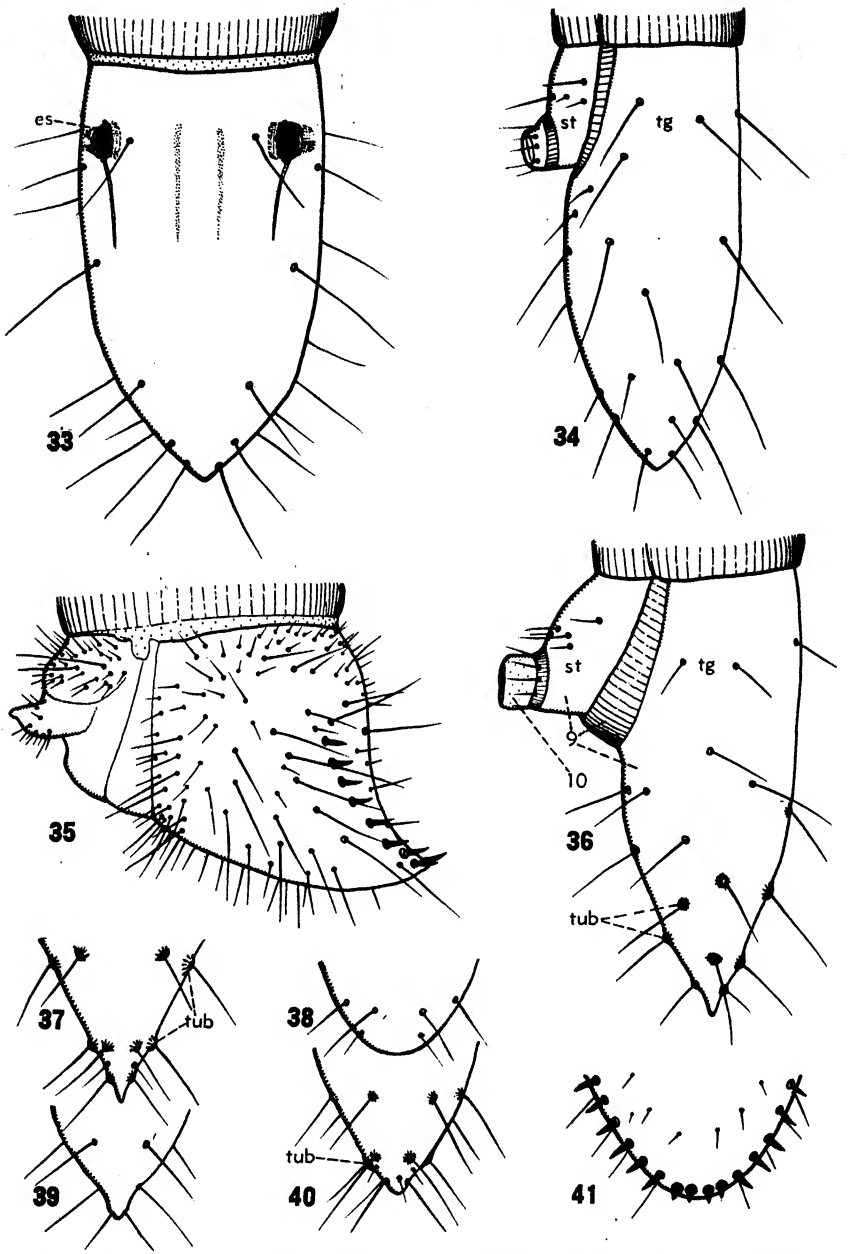


23

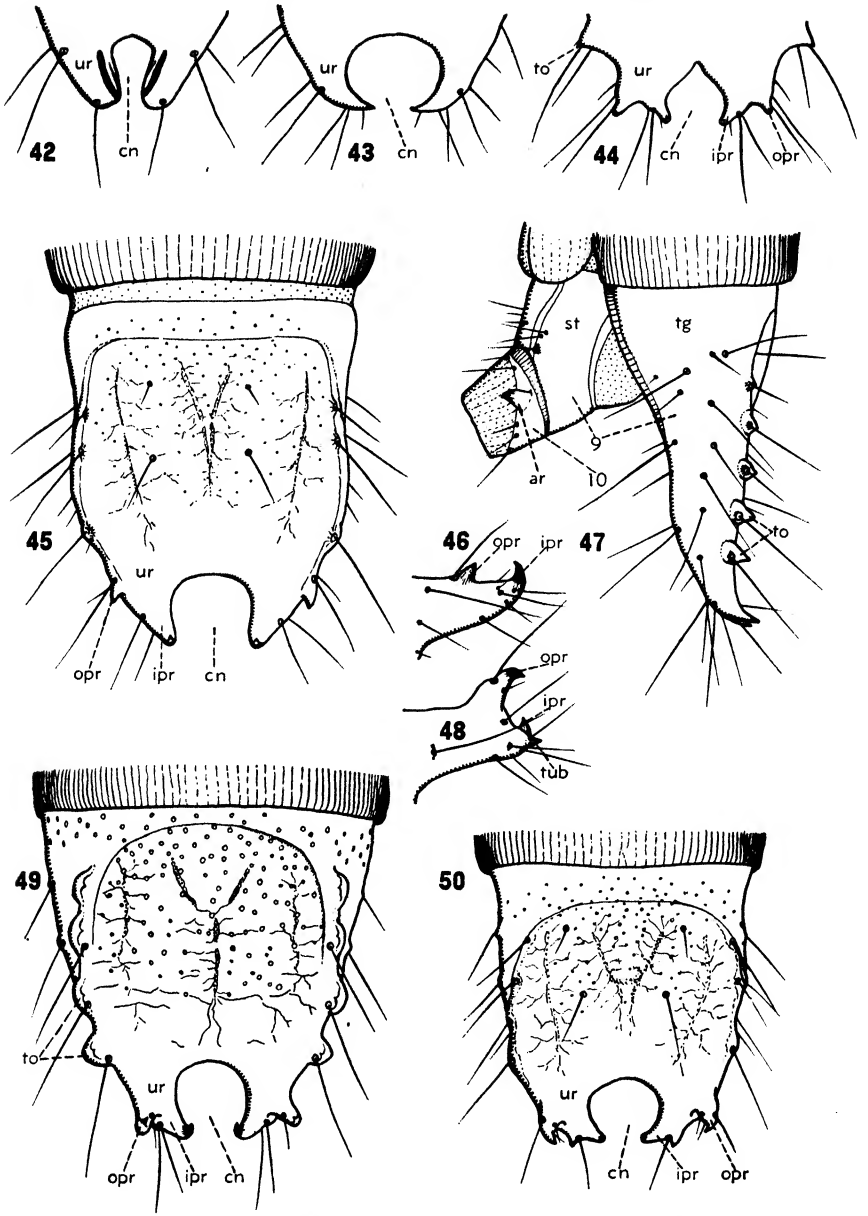
FIGS. 12 TO 23. 12. *Ludius aeripennis destructor, head, dorsal.* 13. *L. destructor, head, ventral.* 14. *Eleodes sp. (Tenebrionidae), head, dorsal.* 15. *Aeolus mellillus, nasale, dorsal.* 16. *Ludius kendalli, nasale, dorsal.* 17. *Ludius limoniiformis, nasale, dorsal.* 18. *Limonius pectoralis, nasale, dorsal.* 19. *Dalopius parvulus, nasale, dorsal.* 20. *Aeolus mellillus, left mandible, dorsal.* 21. *Hypnoidus dubius, left mandible, dorsal.* 22. *Agriotes limosus, left mandible, dorsal.* 23. *Agriotes mancus, left mandible, dorsal.*



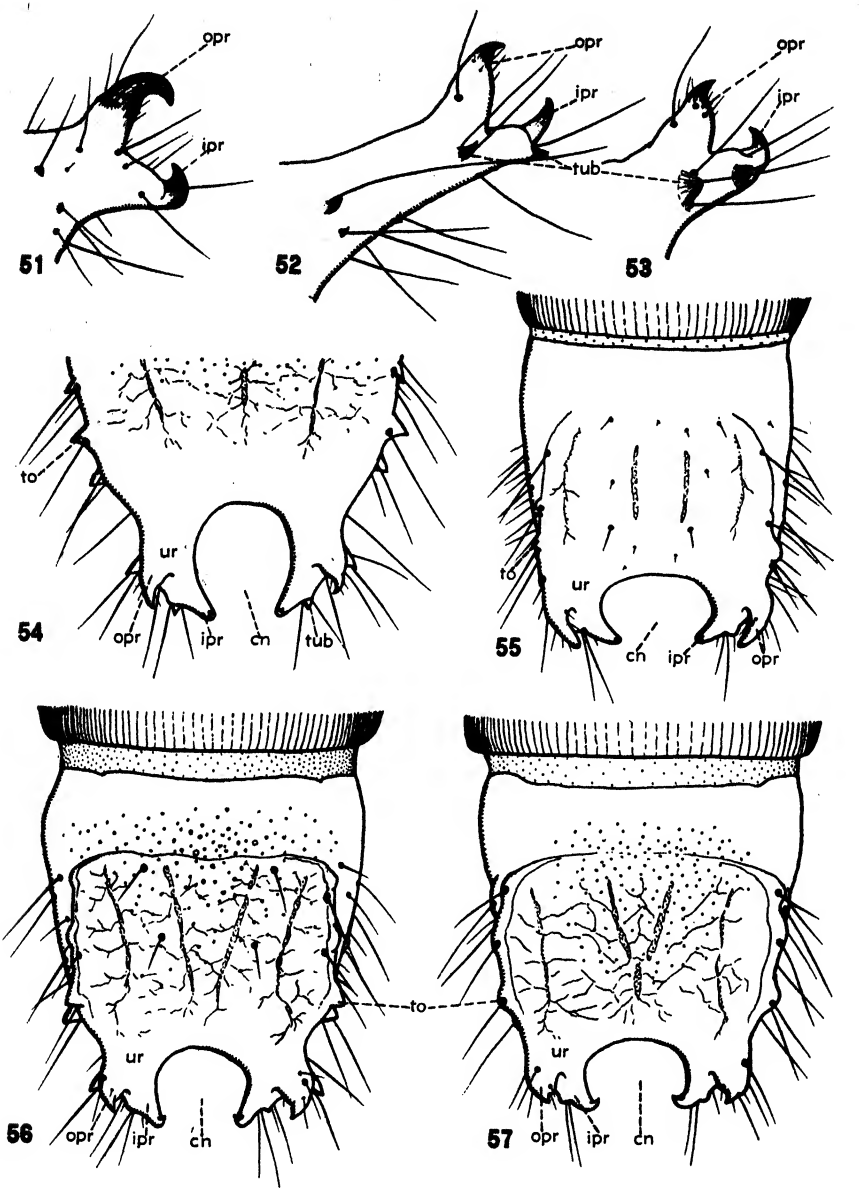
FIGS. 24 TO 32. 24. *Limonius ectypus (?), anterior part of prothorax, ventral.* 25. *Cryptohypnus abbreviatus, head, prothorax, and mesothorax, dorsal.* 26. *Limonius ectypus (?), fifth abdominal segment, dorsolateral.* 27. *Ludius aeripennis destructor, prothorax (legs removed), ventral.* 28. *L. destructor, episternum of mesothorax, lateral, to show spine-like setae.* 29. *Hemicrepidius sp., left half of dorsum of fourth abdominal segment, dorsolateral.* 30. *Melanotus sp., ninth abdominal segment, dorsal.* 31. *Melanotus sp., tip of ninth abdominal segment, lateral.* 32. *Ampedus sp., ninth abdominal segment, dorsal.*



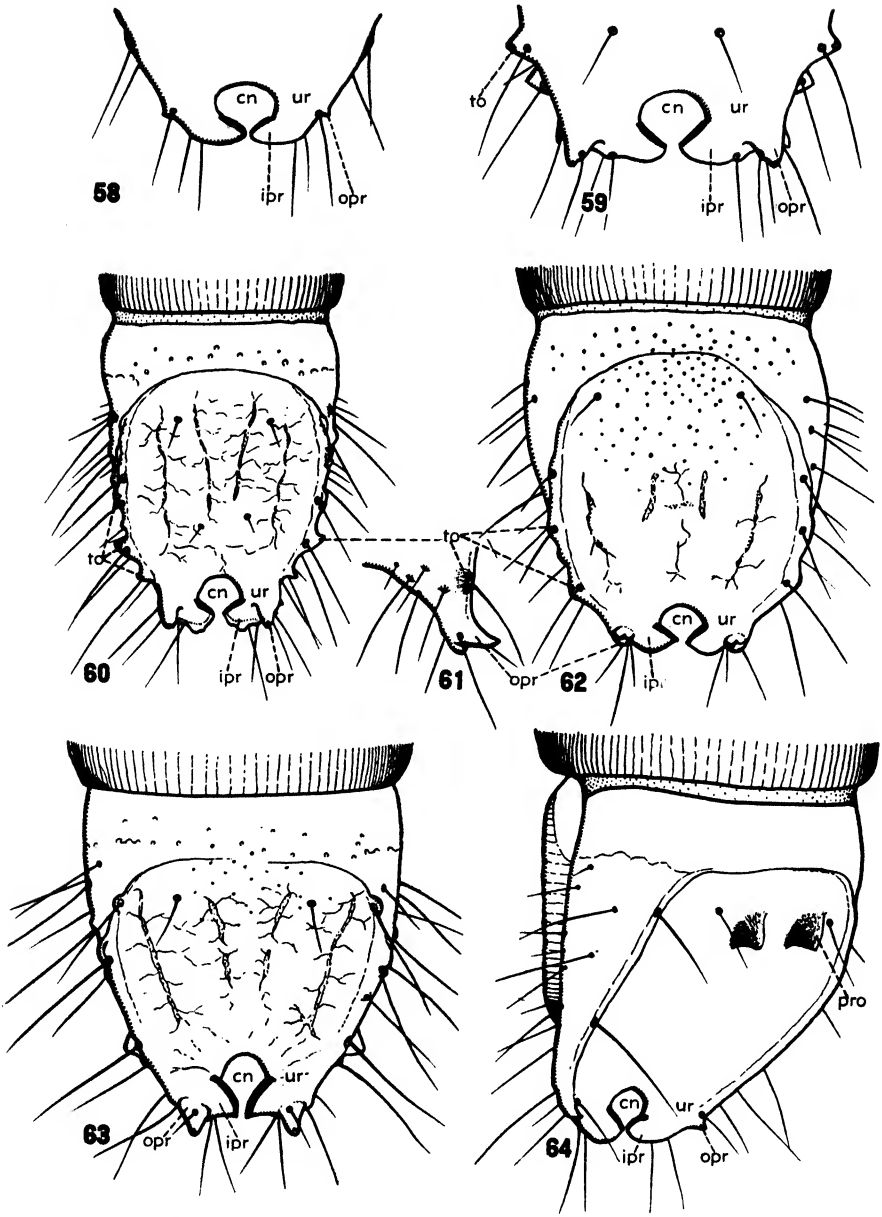
FIGS. 33 TO 41. 33. *Agriotes mancus, ninth abdominal segment, dorsal.* 34. *Agriotes limosus, ninth and 10th abdominal segments, lateral.* 35. *Eleodes sp. (Tenebrionidae), ninth and 10th abdominal segments, lateral.* 36. *Dalopius parvulus, ninth and 10th abdominal segments, lateral.* 37. *D. parvulus, tip of ninth abdominal segment, dorsal.* 38. *Parallelostethus attenuatus, tip of ninth abdominal segment, dorsal.* 39. *Agriotes (?) sp., tip of ninth abdominal segment, dorsal.* 40. *Agriotes criddlei, tip of ninth abdominal segment, dorsal.* 41. *Eleodes sp. (Tenebrionidae), tip of ninth abdominal segment, dorsal.*



FIGS. 42 TO 50. 42. *Eanus decoratus, tip of ninth abdominal segment, dorsal.* 43. *Hypnoidus dubius, tip of ninth abdominal segment, dorsal.* 44. *Aeolus mellillus, tip of ninth abdominal segment, dorsal.* 45. *Cryptohypnus nocturnus, ninth abdominal segment, dorsal.* 46. *C. nocturnus, tip of ninth abdominal segment, lateral.* 47. *Aeolus mellillus, ninth and 10th abdominal segments, lateral.* 48. *Cryptohypnus abbreviatus, tip of ninth abdominal segment, lateral.* 49. *Hemicrepidius memnonius, ninth abdominal segment, dorsal, drawn from larval exuvium of a reared specimen.* 50. *Cryptohypnus abbreviatus, ninth abdominal segment, dorsal.*



FIGS. 51 TO 57. 51. *Ludius sexualis (?), tip of ninth abdominal segment, lateral.* 52. *Ludius glaucus, tip of ninth abdominal segment, lateral.* 53. *Ludius aeripennis destructor, tip of ninth abdominal segment, lateral.* 54. *Ludius glaucus, posterior half of ninth abdominal segment, dorsal.* 55. *Ludius sexualis (?), ninth abdominal segment, dorsal.* 56. *Ludius pruininus, ninth abdominal segment, dorsal.* 57. *Ludius aeripennis destructor, ninth abdominal segment, dorsal.*



FIGS. 58 TO 64. 58. *Limonius sp. (Group 1), tip of ninth abdominal segment, dorsal.* 59. *Ludius cylindriformis (?), tip of ninth abdominal segment, dorsal.* 60. *Ludius limoniiformis (?), ninth abdominal segment, dorsal.* 61. *Limonius ectypus (?), tip of ninth abdominal segment, lateral.* 62. *L. ectypus (?), ninth abdominal segment, dorsal.* 63. *Ludius kendalli, ninth abdominal segment, dorsal.* 64. *Limonius pectoralis, ninth abdominal segment, dorsolateral.*

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. DECEMBER, 1943 NUMBER 12

DRIED WHOLE EGG POWDER

IX. EFFECT OF DRYING CONDITIONS ON QUALITY[1]

BY A. H. WOODCOCK[2] AND MARGARET REID[3]

Abstract

Liquid whole egg was spray dried in a small, laboratory drier at various flow rates of liquid egg and at different inlet and exhaust air temperatures. Quality of the powder, as assessed by chemical methods, palatability, and baking tests, was progressively improved as the exhaust air temperature was lowered. Inlet air temperatures above 107° C. (225° F.) had a deleterious effect. Lowering the drying temperature, however, had an adverse effect on the rate of production.

Introduction

The necessity of producing large quantities of high quality egg powder in a relatively short time has resulted in the operation of egg-drying plants by somewhat arbitrary methods. Apparently little systematic study has been made of factors affecting the production of the maximum quantity of powder compatible with good quality in the cyclonic type of spray drier. Accordingly, experiments were made with a laboratory model spray drier (6) and it is hoped that these will be of some value in guiding commercial egg-drying plants in the production of first grade powder. The factors affecting spray drying of egg powder described in this paper are: inlet and exhaust air temperatures and flow rate of liquid egg.

Materials and Methods

Grade *A* Large eggs obtained from the open market were used exclusively throughout these experiments. The eggs were broken and the whites and yolks thoroughly mixed by mechanical stirring at a low speed to prevent frothing. Chalazae, yolk membranes, and shell membranes were removed by straining through cheese cloth and a fine wire screen.

Three series of measurements were made involving three variables, namely, flow rate of liquid egg into the drier, and inlet and exhaust air temperatures. In each series one of the three variables was held constant while the other two were varied dependently. In the first series the flow rate of the liquid

[1] *Manuscript received September 7, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as paper No. 102 of the Canadian Committee on Food Preservation and as N.R.C. No. 1162.

[2] *Biophysicist, Food Investigations.*

[3] *Biochemist, Food Investigations.*

egg was held approximately constant while the inlet air temperature was varied in 14° intervals from 79° C. (175° F.) to 135° C. (275° F.). In the second series the inlet air temperature was held constant at 121° C. (249° F.) and the flow rate of liquid egg was varied, resulting in a range of exhaust air temperatures. The third series was arranged with inlet temperatures from 66° C. (150° F.) to 135° C. (275° F.) in 14° intervals while the flow rate of liquid egg was adjusted to give an exhaust air temperature of 60° C. (140° F.).

Quality of the powder was assessed by determinations of the moisture content, fluorescence, potassium chloride, and refractometric values, and palatability ratings by methods already described (2, 3, 4) and by baking tests.

The baking quality of the product was assessed by measuring the volume of sponge cakes made from the various powders. These sponge cakes were made with 12 gm. of dried egg, 40 gm. of white sugar, 40 gm. of white flour, and 38 ml. of water. The dried egg and sugar were mixed thoroughly before adding the water, thus preventing the formation of lumps in the mixture. This was then beaten at high speed in a "Mixmaster" for 10 min. After beating, the flour was added with sufficient mixing at the lowest speed to give a homogeneous batter. The cake was baked for 35 min. in an oven at 163° C. (325° F.). The volume of the cake was measured in terms of the volume of rape seed displaced (1).

Results

Table I shows the results of varying the inlet air temperature while the rate of flow of liquid egg was held constant. It may be seen that the exhaust air temperature decreased as the inlet air temperature was lowered but that the drop in temperature was not constant. This might be expected since, in a small experimental drier, the ratio of surface area to volume is high, resulting in appreciable heat loss through the walls.

TABLE I

EFFECT OF INLET AND EXHAUST AIR TEMPERATURES ON THE QUALITY OF SPRAY DRIED WHOLE EGG

Air temperature				Flow rate of liquid egg, ml./min.	Quality measurements					
Inlet		Exhaust			Moisture content, %	Fluorescence value	Potassium chloride value	Refractometric value	Palatability score	Cake volume, ml.
° C.	° F.	° C.	° F.							
134	274	84	184	33.8	1.09	37.3	45.7	234	7	287
122	251	79	175	31.1	1.14	30.6	60.1	248	8	296
107	225	69	156	32.7	2.08	18.8	70.0	266	8	317
94	201	58	137	30.1	2.82	15.2	73.9	267	8½	383
79	175	50	122	27.2	3.08	14.2	78.1	265	8½	434

The moisture contents of the egg powder increase with decreasing air temperatures. This is due to the lower capacity for moisture of cooler air and the slower rate of evaporation.

The quality of the resulting egg powder, as shown by chemical methods, palatability, and cake volume, increased with decreasing air temperatures. Cake volume is also shown in Fig. 1.

Table II indicates the effect of lowering the exhaust air temperature while holding the inlet air temperature constant. It will be seen that lowering the exhaust air temperature considerably increased the rate of production. Again, owing to heat losses through the walls of the drier, the drop in temperature of the air passing through the drier was not proportional to the flow of liquid egg. As the exhaust air temperature was reduced the moisture content of the resulting egg powder was increased. This was due to both the greater absolute amount of moisture to be removed and the lower moisture capacity of the cooler exhaust air.

TABLE II

EFFECT OF EXHAUST AIR TEMPERATURE ON THE QUALITY OF SPRAY DRIED WHOLE EGG

Air temperature				Flow rate of liquid egg, ml./min.	Quality measurements					
Inlet		Exhaust			Moisture content, %	Fluorescence value	Potassium chloride value	Refractometric value	Palatability score	Cake volume, ml.
° C.	° F.	° C.	° F.							
121	249	76	168	35.1	1.18	28.8	56.3	248	8	284
121	249	71	160	43.1	1.59	28.0	57.6	254	8	412
121	249	67	152	52.6	1.81	23.1	60.4	256	8	418
121	249	62	144	64.4	3.71	20.2	59.8	262	8½	421

Quality as determined by fluorescence and refractometric values and cake volume (Table II) showed a regular increase in quality as the exhaust air temperature was lowered. The potassium chloride values and palatability ratings were somewhat irregular. All these criteria however showed a definite trend toward a higher quality product when the exhaust air temperature was lowered.

Table III shows the effect of lowering the inlet air temperature while maintaining a constant exhaust air temperature. The effect on production

TABLE III

EFFECT OF INLET AIR TEMPERATURE ON THE QUALITY OF SPRAY DRIED WHOLE EGG

Air temperature				Flow rate of liquid egg, ml./min.	Quality measurements					
Inlet		Exhaust			Moisture content, %	Fluorescence value	Potassium chloride value	Refractometric value	Palatability score	Cake volume, ml.
° C.	° F.	° C.	° F.							
121	250	60	140	59.5	1.93	17.1	64.7	261	8	420
107	225	60	140	47.5	2.01	17.4	63.4	264	8½	444
93	200	60	140	32.6	1.78	19.2	63.7	258	8½	453
79	175	60	140	16.4	2.05	17.6	70.3	258	8	447

was very marked and again the drop in air temperature indicated loss of heat through the walls of the drier. Altering the inlet air temperature appears to have little effect on the moisture content. Two counteracting phenomena caused this: with higher inlet air temperatures more moisture was evaporated with consequently moister exhaust air, and at the higher temperatures evaporation was more rapid.

The quality measurements in general showed irregular changes, the differences being hardly distinguishable from experimental errors. When the temperature was reduced from 124° C. (250° F.) to 107° C. (225° F.) the fluorescence and potassium chloride values showed no significant change. However, quality as tested by the refractometric value, palatability, and especially cake volume, however, did show some improvement. Further lowering of the inlet air temperature effected little change in quality until a temperature of 79° C. (175° F.) was reached. At this temperature there was suggestion of a decrease in quality. It is probable that this is due to the very low flow rate of the liquid egg. When the rate is reduced, a greater proportion of the egg powder temporarily sticks to the walls of the drier and suffers some thermal deterioration.

Discussion

From the above results it will be seen that the drop in temperature of the air passing through the drier is dependent on the rate of production. In full scale commercial driers where the loss of heat through the walls is negligible the drop in temperature is essentially proportional to the rate of production. It is therefore advisable in order to secure maximum production to use the highest inlet air temperature and lowest exhaust air temperature compatible with the quality desired.

The drop in temperature desired in drying egg is dependent on good initial quality and low moisture content. The moisture content has been shown to be closely connected with the keeping quality (5). Lowering the outlet temperature has been shown in Tables I and II to increase the moisture content.

Initial quality has been shown to be directly related to exhaust air temperature. This effect is not so marked in the second series of experiments (Table II) as in the first (Table I), since the range of exhaust air temperatures is much less in the second series. Table I indicates that exhaust temperatures below 60° C. (140° F.) were required in order to obtain first quality powder. Table III indicates that the maximum inlet air temperature for first quality powder was between 107° C. (225° F.) and 121° C. (250° F.).

While these data have been obtained on an experimental drier they should be generally applicable to commercial plants since exposure of egg particles to warm air is the primary factor in drying. Some variation in their actual magnitudes can, however, be expected, particularly in moisture content.

PLATE I

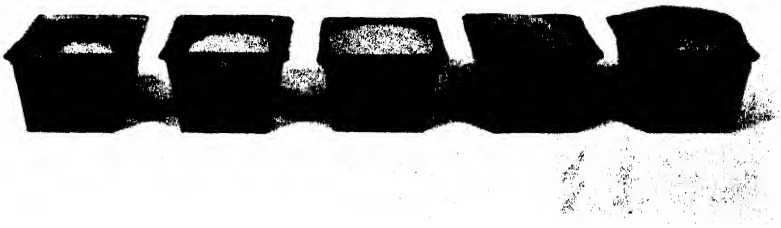


FIG. 1. *Increase in cake volume with decrease in drying temperatures.*

Acknowledgment

The authors wish to express their thanks to Mr. H. Tessier, Laboratory Assistant, for his valuable technical aid in carrying out the drying operations.

References

1. MALLOCH, J. G. and COOK, W. H. Cereal Chem. 7 : 307-310. 1930.
2. PEARCE, J. A., THISTLE, M. W., and REID, M. Can. J. Research, D, 21 : 341-347. 1943.
3. THISTLE, M. W., PEARCE, J. A., and GIBBONS, N. E. Can. J. Research, D, 21 : 1-7. 1943.
4. WHITE, W. H. and GRANT, G. A. Can. J. Research, D, 21 : 203-210. 1943.
5. WHITE, W. H. and THISTLE, M. W. Can. J. Research, D, 21 : 211-222. 1943.
6. WOODCOCK, A. H. and TESSIER, H. Can. J. Research, A, 21 : 75-78. 1943.

THE DEHYDRATION OF PORK[1]

By Jesse A. Pearce[2]

Abstract

Four preprocessing and three drying procedures for the dehydration of pork were studied. The best product resulted from cooking in an open steam-jacketed kettle, followed by mincing and tunnel–tray drying in four hours or less with a tray load of 2 lb. per square foot. Little difference in quality of the product was observed for air velocities of 700 to 1200 ft. per min. over the trays, or for initial stage temperatures between 70° and 80° C. and final stage temperatures between 55° and 70° C.

Pork, when dried to a moisture content of 3 to 4% in less than four hours, still retained a high percentage of the thiamin present in the raw meat, showed no fat deterioration when assessed by peroxide oxygen determinations, and on reconstitution had a palatability rating approximately equal to that of the initial cooked material.

Introduction

General interest has been aroused in the dehydration of food products as a means of conserving shipping space and packaging materials. In addition, dried foodstuffs show greater resistance to spoilage during storage and transport. This study was confined entirely to the dehydration of pork, as pork is the only meat that is produced in substantial quantities in Canada at the present time. When these studies were undertaken little information was available on pork dehydration. This paper describes the effect of various preprocessing treatments and methods of dehydration on the quality of dried pork.

Materials

Uncured sow ham was used for the greater part of this investigation, as it was felt that this material was representative of the type of pork likely to be dried. In addition, separation of meat from fat and bone is more easily and cheaply accomplished in the ham than in other cuts. Some export cured ham was also dried for comparison with the uncured dried product.

Precooking Procedure

Initially, uncured sow ham was tunnel–tray dried under four conditions: coarsely minced meat, and meat in chunks of approximately 2 cu. in., were dried both with and without precooking. The results are shown in Table I. Although the drying conditions were found to be impractical in subsequent work, Table I indicates that, for drying purposes, cooked minced meat was a superior starting material. In addition, it was believed that dried chunks would cause packaging difficulties; therefore cooked minced meat was finally

[1] *Manuscript received August 26, 1943.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 103 of the Canadian Committee on Food Preservation and as N.R.C. No. 1172.

[2] *Biochemist, Food Investigations.*

chosen as the most suitable material for drying, in spite of the significant decrease noted in the palatability score.

TABLE I

EFFECT OF METHOD OF PREPARATION ON DRYING RATE AND FINAL QUALITY

Temperature, 93° C.; air flow, 900 ft. per min.; tray load, 2 lb. per sq. ft.

Condition of meat	Relative humidity of tunnel air (%)			Over-all drying rate (% per hr.)	Drying time (hr.)	Quality of dried product			
	Initial	Drying 2/3 complete	Final			Moisture content (%)	Palatability score		Peroxide oxygen value (ml. 0.002 *N* thiosulphate)
							Initial	After drying	
Chunks, raw (1" × 1" × 2")	53	22	21	10	9	15	7.5	6.6	13
Chunks, raw[1] (1" × 1" × 2")	68	34	32	9	10	21	7.5	6.1	5.4
Chunks, cooked (1" × 1" × 2")	47	22	18	10	8	23	7.5	6.9	14
Minced, raw	50	24	29	13	6.5	24	7.8	7.4	11
Minced, cooked	66	27	24	12	8	2.0	7.8	5.3	4.2
Minced, cooked[1]	60	33	35	13	7.5	2.0	7.9	4.0	0

[1] *Smoke added for two hours at the commencement of drying.*

Throughout this work pork was considered to be sufficiently precooked when redness had just disappeared from the centre of meat chunks or of a mass of minced meat. Meat was minced using a Hobart grinder with a $\frac{3}{8}$ in. plate.

Four precooking procedures were investigated. The first method involved separating the meat from bone, fat, and rind, mincing, and cooking for three minutes per pound at 15 lb. pressure. Meat prepared in this way was very wet, dripped badly in the initial stages of drying, and dried slowly, forming large, spongy aggregates in the process. A test sample of meat prepared by this method retained 83% of the thiamin present in the raw meat.

The second method involved cutting the whole ham to the bone at 3- or 4-in. intervals, and cooking at 93° to 104° C. (200° to 220° F.) in a retort, allowing 20 min. per pound. The meat was then separated from bone, fat, and rind, minced, and dried. Although export cured ham could be handled by this method, the greater bulk and heavier fat layers of the larger sow ham prevented its satisfactory use. Preparation of meat in this manner avoided the formation of spongy aggregates during the drying process.

The third procedure involved separating the meat from bone, fat, and rind, and cutting it into slices 1 in. thick, each weighing about a pound. These slices were placed on trays to a depth of about 4 in. and cooked in a retort for 20 to 30 min. at 15 lb. steam pressure. The cooked slices were removed

and minced. The liquor separating from the meat was collected and concentrated to about one-quarter its original volume and mixed with the mince before drying.

The fourth method, open pot cooking, eliminated separate concentration of liquor. A steam-jacketed kettle (15 lb. steam pressure in the jacket) replaced the trays and retort. The meat was cooked without added water and with constant stirring (allowing about one minute per pound of meat). Pork cooked by this method was observed to contain 87% (σ = 6%) of the thiamin present in the raw material.

Moisture contents and palatability scores of pork, prepared for drying by each of the different methods, are compared in Table II. It is evident that there is no significant difference in the palatability of pork cooked by any method studied. The last procedure was considered the most practical for dehydration purposes because of the lower moisture content of the material going into the drier.

TABLE II

MOISTURE CONTENTS AND ORGANOLEPTIC RATINGS OF PORK PREPARED FOR DRYING BY DIFFERENT METHODS

Method of preparation	Moisture content (%)		Palatability score
	Mean	Standard deviation	
Raw pork	71	4	
Cooked chunks			8.3
Minced pork, pressure cooked	68	3	7.6
Whole, cured ham cooked, then minced	66	2	8.3
Pork chunks, pressure cooked, liquors concentrated and added back to minced meat	61	3	8.1
Pork chunks, cooked in open kettle and minced	56	3	8.3

Method of Drying

Three types of drying apparatus, representing those most likely to be available for commercial purposes, were studied.

Dried pork was produced by vacuum (26 in. gauge)–tray drying at 71° and 82° C. (160° and 180° F.), with a loosely spread meat load of 2 lb. per square foot.

Investigations were also carried out using a laboratory model atmospheric, double-drum drier. The drums were 6 in. in diameter and the meat was in contact with the drums for two-thirds of one revolution. Steam pressure in the drums was varied between 15 and 60 lb. (gauge); speed of rotation from 0.25 to 3.0 r.p.m.; and spacing between the drums between $\frac{1}{16}$ and $\frac{1}{64}$ in.

Experimental tunnel driers were used for the greater part of the investigation. The smaller laboratory tunnel–tray drier accommodated 12 sets of trays 6 × 8 × 1 in. The trays were arranged in three tiers with 1 in. intervals between them to allow free passage of the air over the meat. For convenience

in measuring the weight decrease without interrupting the drying process, the set of trays rested on the platform of a scale. Temperatures of drying were varied between 49° and 93° C. (120° and 200° F.). The air velocities used were 300, 600, and 900 ft. per min. in the open tunnel, corresponding to 400, 800, and 1200 ft. per min. over the trays. High humidities were obtained by closing the tunnel, and low humidities by changing the air in the drier 15 or 16 times per min. on a volume basis. Reduction of the meat load from 2 to 1 lb. per square foot and stirring the meat were tried in an attempt to reduce the time of drying. Smoke was added in some cases during drying, with the hope of retarding deterioration in the fat (4).

Following favourable indications obtained with this small tunnel drier, drying by this method was attempted on a larger model. The larger tunnel drier accommodated 28 trays 12.5 × 20 × 1 in., arranged in four stacks of seven tiers. Again 1 in. spaces were allowed for passage of air. The air flow in the open tunnel was 500 ft. per min. which corresponded to 700 ft. per min. over the trays. The air in the tunnel was changed about 15 times per min.

Analytical Methods

Most of the quality measurements at present used on dried foods are considered to be inadequate. Fat content was considered to be of minor importance since it was felt that hams could not easily be trimmed to any given fat content. The fat content of the trimmed meat averaged 31% (σ = 9%) on a dry matter basis. For dried pork the features believed to have the greatest significance were palatability, the quality of the fat, and amount of thiamin retained.

Moisture Content.—Moisture content was determined by vacuum drying 5-gm. samples for 16 hr. at 100° C.

Fat Content.—Petroleum-ether–soluble materials were used as a measure of the fat content. Fifty-gram samples were extracted in a Soxhlet for 16 hr.

Peroxide Oxygen Value.—This determination had been found satisfactory for deterioration in pork fat (1). The method has been described (3). The value observed for the initial material, whether raw or cooked, was zero ml. 0.002 *N* thiosulphate per gram of extracted fat.

Thiamin Content.—A method of determining thiamin in meat and meat products (2) proved satisfactory for the present work. Five-gram samples of raw or cooked materials and 2-gm. samples of dried materials were used. For convenience, the results are given as percent of thiamin retained in the dried product, calculations being based on the amount present in the initial cooked material (*D.M.B.*).

Palatability Score.—The reconstituted samples were given organoleptic ratings by a panel of 14 tasters. Panel members tasted the unsalted, reconstituted product and scored as follows: 10, for excellent fresh pork; 8, fair fresh pork; 6, poor pork that could be eaten if necessary; 4, definitely unpleasant; 2, repulsive. (Intermediate scores and zero could be applied.)

It was observed that the necessary difference for significant change in the edibility of the meat varied from about 0.8 to 1.2 palatability units, at the 5% level of statistical significance. Therefore, in making general comparisons, differences greater than 1.0 palatability units are considered significant.

Results

Vacuum–Tray Drying

The conditions of vacuum–tray drying and the results of analyses on the dried product are given in Table III. Drying was considered complete when the rate of loss reached about 0.2% per hour. The extended drying period, high peroxide oxygen values for the fat, decrease in edibility, and retention of only a small fraction of the thiamin originally present indicated the impracticability of this type of drying. Analysis of the distillate showed the presence of 48% of the thiamin originally present in the cooked meat. As the distillate had the odour and flavour of a fairly good meat broth, a large proportion of the materials contributing to flavour were believed to be lost during drying.

TABLE III

VACUUM–TRAY DRYING—MINCED SOW HAM COOKED (UNCURED)

Vacuum, 26 in. gauge; tray load, 2 lb. per sq. ft.

Dry bulb temp.	Over-all drying rate (% per hr.)	Drying time (hr.)	Moisture content (%)	Quality of the dried product			
				Peroxide oxygen value	Palatability score		Thiamin retained (%)
					Initial	After drying	
71° C. (160° F.)	3	30	24	22	7.9	6.5	
82° C. (180° F.)	4	23	9.1	6.7	7.9	7.1	21

Atmospheric Double-Drum Drying

Drum drying yielded a flaky product that on reconstitution failed to reabsorb as much water as samples prepared by the other methods. Analyses of the dehydrated material (Table IV) indicated that it was difficult to obtain a really dry product. In many instances, the fat present in dried material had excessive peroxide oxygen values. Occasional palatability tests showed the reconstituted product to have a poor texture although it had a not unpleasant resistance to chewing.

Tunnel–Tray Drying—Small Drier

As there was little information available on the dehydration of pork, it was assumed initially that tunnel drying demanded relatively high humidities and small initial temperature increments to minimize case hardening and deterioration of the fat. Table V shows such conditions of drying and quality measurements for uncured sow ham and export cured ham. Drying was considered

TABLE IV

DOUBLE DRUM DRYING—MINCED SOW HAM COOKED (UNCURED)

Steam pressure in drums (lb. gauge)	Drum speed (r.p.m.)	Space between drums (in.)	Approximate rate of processing (lb. per hr.)	Quality of dried product				
				Moisture content (%)	Palatability score		Peroxide oxygen value	Thiamin retained, (%)
					Initial	After drying		
15	3.00		36	51			25	
30	3.00		36	44	7.9	7.1	14	
30	1.50	1/64	11	33			28	97
30	0.25	1/32	2	15	8.3	7.0	1.0	60
60	1.50	1/64	14	18			4.6	87
60	1.50	1/32	21	28			3.7	81
60	1.50	1/16	21	31			2.6	81
60	0.25	1/64	2	2.9	8.3	6.0	1.9	20

TABLE V

TUNNEL–TRAY DRYING—MINCED HAM, COOKED

Tray load, 2 lb. per sq. ft.

Material	Dry bulb temperature	Relative humidity of tunnel air (%)			Air flow (ft./min.)	Overall drying rate (% per hr.)	Drying time (hr.)	Quality of the dried product				
		Initial	Drying 2/3 complete	Final				Moisture content (%)	Palatability score		Peroxide oxygen value	Thiamin retained (%)
									Initial	After drying		
Uncured sow ham	93° C. (200° F.)	56	27	24	900	12	8	2.0	7.8	5.3	4.2	16
	82° C. (180° F.)	50	31	27	900	11	9	4.8	7.8	7.6	7.4	21
	71° C. (160° F.)	64	34	30	900	10	10	7.4	7.0	7.2	7.8	26
	71° C. (160° F.)	40	11	8	600	11	9	2.9	6.2	6.2	29	
	71° C.[1] (160° F.)	64	40	32	900	14	7	6.5	7.9	7.6	16	
	71° C.[1] (160° F.)	72	53	38	900	23	4	17	6.2	6.4	17	
	60° C. (140° F.)	52	22	20	900	9.3	10	5.7	7.2	7.2	23	
Export cured ham	71° C. (160° F.)	54	46	44	300	12	8	5.7	8.8	7.5	8.3	
	71° C. (160° F.)	62	38	38	600	11	8.5	9.9	8.7	8.0	14	
	71° C. (160° F.)	54	40	39	900	12	8	3.8	8.8	7.9	10	
	71° C.[2] (160° F.)	64	47	48	900	13	7	12	8.6	7.9	17	
	93° – 71° C.[3] (200–160° F.)	57	24	33	900	15	6	19	8.6	8.6	3.4	

[1] *Drying stopped arbitrarily.*

[2] *Meat stirred on trays every two hours.*

[3] *Higher temperature for four hours, low temperature for remainder of period.*

complete when the rate of weight loss reached about 0.2% per hour. During drying, the humidity dropped rapidly for the first two-thirds of the period and slowly for the remainder of the time.

Although drying by this procedure could not be considered satisfactory, the results yielded information of value. Comparison of the palatability of the dried products obtained from the two types of ham indicated that export cured ham suffered greater deterioration than sow ham. The optimum drying temperature seemed to be 71° C. (160° F.). Different air velocities had no pronounced effect on the rate of drying. Stirring the meat during drying effected no improvement either in drying time or quality of product.

The addition of smoke to the air stream for two hours at the commencement of the run reduced the peroxide oxygen values observed in the fat, but resulted in decreased palatability scores (Table I).

It appeared advisable at this stage to determine the behaviour of the fat and the temperature changes occurring in the meat during a typical drying run. Table VI shows the data obtained when peroxide oxygen values of the fat were measured at intervals during drying. These results indicated that from the point of view of fat quality the drying period should be no greater than four hours. However, the peroxide oxygen measurement, when applied to fat extracted from dehydrated pork was unsuitable as an indication of eating quality (Table V).

TABLE VI

CHANGE IN PEROXIDE OXYGEN VALUE OF FAT DURING TUNNEL–TRAY DRYING

Temperature, 71° C. (160° F.); air flow, 900 ft. per min.

Time (hr.)	Peroxide oxygen value
Uncooked fat	0
Cooked fat (zero time)	0
2.0	0
4.0	0
5.0	1.9
6.0	4.2
7.0	4.6
8.0	6.7

Fig. 1 shows the temperature changes occurring throughout the meat as drying proceeds. It is evident from these results that variations in temperature existed within trays of meat as well as between trays. Evaporation from the wet meat caused a rapid drop in air temperature. The over-all temperature drop, with an air flow of 1200 ft. per min., over four columns of trays of meat, loaded to 2 lb. per square foot, was about 11° C. (20° F.). It was evident from the fact that the meat assumes the temperature of the wet bulb at the start of drying that much higher temperatures might be used initially.

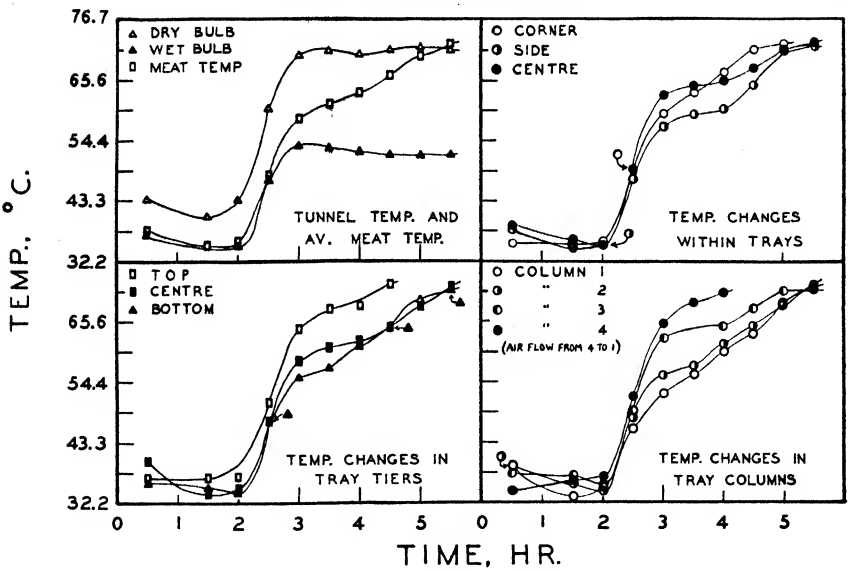


FIG. 1. *Temperature changes occurring in trays of minced pork during dehydration.*

As a result of the above observations the method of tunnel–tray drying was modified and applied only to uncured sow ham. Methods of cooking that effectively reduced the moisture content were used (cf. Table II). In general, the data obtained (Table VII) indicate that drying at temperatures of about 54° C. (130° F.) and with low relative humidities results in a dried product of better quality as assessed by objective measurements. The use of temperatures as high as 82° C. (180° F.) for a short period at the start of drying caused no marked deterioration in the product. Reduction of the meat load from 2 to 1 lb. per square foot decreases the drying time only slightly. For drying under conditions studied here, an air flow of as low as 300 ft. per min. would greatly reduce the output, but either 600 or 900 ft. per min. would be satisfactory. Pronounced increase in the amount of thiamin retained in the dried product resulted from the use of these shorter drying times and lower drying temperatures (cf. Tables V and VII). Samples of the material dried at 54° C. (130° F.) and of the initial material, when submitted to the tasting panel, were scored 7.9 and 8.3 palatability units respectively, the difference being quite insignificant.

It was observed that the cooked product must be introduced into the drier immediately after cooking and mincing, as allowing the cooled meat to stand resulted in the development of off-odours and flavours that persisted in the dried product, rendering it unpalatable when reconstituted. When holding for short periods was necessary, 93° C. (200° F.) was found to be a convenient and satisfactory temperature.

TABLE VII

TUNNEL–TRAY DRYING—COOKED MINCED SOW HAM (UNCURED)

Conditions under study	Dry bulb temperature	Relative humidity of tunnel air (%)			Meat load (lb./ ft.)	Air flow (ft./ min.)	Overall drying rate (% per hr.)	Quality of dried product		
		Prior to run	Initial	Final				Moisture content (%)	Peroxide oxygen value	Thiamin retained (%)
Drying time—four hours										
Variation in temperature and humidity	93° C. (200° F.)	6	8	9	2	900	25	2.0	11	60
	93° C. (200° F.)	7	17	6	2	900	25	2.0	16	63
	71° C. (160° F.)	6	19	7	2	900	24	3.9	8.4	71
	71° C. (160° F.)	18	35	15	2	900	24	5.7	12	70
	49° C. (120° F.)	16	50	17	2	900	23	13	0	70
	49° C. (120° F.)	26	65	50	2	900	18	30	8.8	75
Drying time—three hours										
Variation in temperature and meat load	54° C. (130° F.)	19	40	19	1	900	32	3.8	1.0	97
	54° C. (130° F.)[1]	29	60	46	2	900	30	13	0	72
	60° – 49° C.[2] (140° – 120° F.)	16	32	24	1	900	30	12	1.2	52
	60° – 49° C.[2] (140° – 120° F.)	14	36	24	2	900	31	8.3	0	89
	71° – 49° C.[2] (160° – 120° F.)	11	27	23	1	900	32	5.7	0	74
	71° – 49° C.[2] (160° – 120° F.)	13	30	22	2	900	32	4.8	3.9	68
	82° – 54° C.[3] (180° – 130° F.)	10	18	45	1	900	32	5.7	0	64
Variation in air flow	82° – 54° C.[3] (180° – 130° F.)	9	30	21	2	900	31	11	0	75
	82° – 54° C.[3] (180° – 130° F.)	18	34	46	2	600	31	9.1	0	91
	82° – 54° C.[1,3] (180° – 130° F.)	20	24	37	2	300	26	26	0	88

[1] *Stirring at half hour intervals gave results no different from those recorded.*
[2] *Two hours at the higher air temperature.*
[3] *One hour at the higher air temperature.*

Tunnel–Tray Drying—Large Drier

The favourable results obtained with the small drier led to an attempt at drying pork on a larger scale. It was hoped that conditions similar to those described above would be satisfactory for the larger drier. Fresh sow ham was cooked and minced before use in this apparatus and the tray load was 2 lb. per square foot of loosely spread meat.

The first experiment was an attempt to dry approximately 100 lb. of trimmed meat (pressure cooked, method three) as a single charge. The dry bulb temperature during the first two hours of drying was 77° C. (170° F.)

and during the last two hours 51° C. (124° F.). The relative humidity during the first two hours dropped from 60 to 37% and during the last two hours from 64 to 46%.

This experiment was unsuccessful (Tables VIII and IX) as the moisture content of the final product was too high. Also, the time involved in mincing and loading the trays with this quantity of meat was of sufficient length to allow the meat to cool and develop off-odours.

TABLE VIII

CHANGES IN WEIGHT AND AMOUNT OF MOISTURE IN MEAT LOAD PER TRAY DURING DRYING

Drying time	Change in weight of meat load (lb.)		Apparent percentage of total moisture removed calculated from loss of weight	
	Experiment one	Experiment two	Experiment one	Experiment two
Initially	3.70	3.70	—	—
One hour	2.73	2.52	43.8	64.8
Two hours	1.83	2.10	84.6	88.0
Three hours	1.65	1.90	92.8	99.0
Four hours	1.55	1.76	97.2	106.6
	Actual moisture loss		92.0	96.1

TABLE IX

QUALITY OF INITIAL AND DRIED PORK

Characteristic	Experiment one		Experiment two		
				Dried	
	Initial	Dried	Initial	Mean	Standard deviation
Moisture content (%)	59.7	10.7	49.2	3.8	0.5
Fat content (%, *D.M.B.*)				31.6	5.4
Organoleptic rating			8.6	8.5	0.2
Peroxide oxygen value	0	0	0	0	
Thiamin retained (%)[1]		98.4		91.0	
Thiamin content (γ/gm. *D.M.B.*)				30.2	9.5

[1] *As per cent of that present in cooked, minced meat.*

In the second experiment approximately 100 lb. of meat (open-pot cooked) was dried in 25 lb. lots. The lots were introduced into the drier at hour intervals, one column of trays being loaded at a time. To reduce the temperature of the air passing over the partially dried charge the fresh lot was introduced between the partially dried meat and the hot air delivery. The temperature conditions in the initial stages of drying during the second

experiment were: dry bulb, 81° C. (178° F.); wet bulb, 43° C. (110° F.). During the period when no more meat charges were introduced, a dry bulb temperature of 71° C. (160° F.) and a wet bulb temperature of 37° C. (98° F.) were used. The reduced temperature was selected as equivalent to the reduction in temperature caused by the introduction of fresh meat and difference in position of the charges in the tunnel. The average loss in temperature across a column of trays during the first hour of drying was 6° C. (10° F.); during the second hour, 2° C. (4° F.); and during the third and fourth hours no loss could be detected by the methods used. The loss in temperature occurring in the tunnel was 0.2° C. (0.3° F.) per foot of tunnel length.

Table VIII shows the average change in weight of a tray of meat during drying by each method and the apparent loss in moisture, expressed as a percentage of the moisture present in the cooked meat. The greater actual weight loss in the meat dried in the first experiment is a result of the slightly higher moisture content of pressure cooked meat. The percentage moisture loss calculated on weight changes during drying is greater than the actual decrease determined by moisture analyses. This difference had been observed in earlier work and was believed to be due to the loss during drying of volatile components other than water.

Table IX records the results of analyses of the initial and final product. The product obtained in the second experiment was of excellent quality, although 27% of the thiamin was destroyed during the cooking process. This was greater than the loss (approximately 13%) observed in previous experiments.

These results indicate that drying conditions found satisfactory for the small tunnel drier could be applied when a larger drier was used providing mincing is followed by rapid introduction of meat into the drier.

In general, pork can be successfully tunnel dried to yield a product that when reconstituted is as palatable as the initial material. However, investigation of drying methods is continuing.

Acknowledgments

The author wishes to express thanks to Dr. A. H. Woodcock, Messrs. T. A. Steeves, and E. A. Chadderton for their assistance in constructing driers and to Messrs. R. T. Legault and K. McDonald for their assistance in operating the driers.

References

1. Cook, W. H. and White, W. H. Can. J. Research, D, 19 : 53-60. 1941.
2. Harris, L. J. and Wang, Y. L. Biochem. J. 35 : 1050-1067. 1941.
3. White, W. H. Can. J. Research, D, 19 : 278-293. 1941.
4. White, W. H., Gibbons, N. E., Woodcock, A. H., and Cook, W. H. Can. J. Research, D, 20 : 263-275. 1942.

PARASITES OF TROUT IN ALGONQUIN PROVINCIAL PARK, ONTARIO[1]

By D. A. MacLulich[2]

Abstract

The parasitism of trout in Algonquin Park, Ont., was studied during the spring and summer of 1939 to determine the distribution and abundance of parasites in the several species of trout. The sampling included 34 lakes from five different river drainages.

A list of the parasites follows with the hosts indicated by the letters, *C* for *Cristivomer namaycush* Wahlbaum, *S* for *Salvelinus fontinalis* Linnaeus, and *F* for *Salmo fario* Linnaeus. Protozoa (cysts in kidneys),—*CSF*; trematodes: *Crepidostomum farionis*,—*CS*, *Azygia angusticauda*,—*C*, *Clinostomum complanatum*,—*S*, *Neascus* sp. (larvae),—*S*; cestodes: *Diphyllobothrium* sp. (larvae),—*C*, *Eubothrium salvelini*,—*CS*, *Proteocephalus ambloplitis* (larvae),—*CS*, *Proteocephalus parallacticus*,—*CSF*, *Proteocephalus pusillus*,—*C*; nematodes: *Cystidicola stigmatura*,—*CS*, *Philonema* sp.,—*C*, unidentified larval nematodes,—*CS*, acanthocephala: *Leptorhynchoides thecatus*,—*C*; copepoda: *Salmincola edwardsii*,—*S*, *Salmincola siscowet*,—*C*.

Two of the tapeworms, the two copepods, and the protozoan kidney cysts were generally distributed. The other parasites showed local differences in abundance. Frequency distribution studies of several of the parasite populations indicated that the parasites are not distributed randomly to the hosts.

Introduction

The parasitism of trout in Algonquin Park, Ont., was studied during the spring and summer of 1939. The purposes of the investigation were to determine the distribution and abundance of parasites in the several species of trout. This information was desirable for fish cultural work in the Park waters. Statistical studies of parasite populations from an ecological standpoint are scarce and attention was directed to frequency distributions of several of the parasite populations. The study of the structure of parasite populations is a field of ecology that will repay further investigation and might yield important methods of management.

The data, showing in detail the lakes sampled and the abundance of each parasite in the trout, are deposited with the Royal Ontario Museum of Zoology. Frequency distribution tables of microsporidian, *Diphyllobothrium*, *Eubothrium*, *Proteocephalus*, and copepod populations are available in the same place.

Material

A full examination was made of 177 lake trout (*Cristivomer namaycush* Wahlbaum), 18 speckled trout (*Salvelinus fontinalis* Linnaeus), and one brown trout (*Salmo fario* Linnaeus). In addition, 423 lake and 94 speckled trout were partially examined for certain parasites such as copepods, cysts of

[1] *Manuscript received in original form May 12, 1942, and as revised, October 14, 1943.*

Contribution from the Ontario Fisheries Research Laboratory, Department of Zoology, University of Toronto, Toronto, Ont.

[2] *At the time, Ranger on the Staff of Algonquin Provincial Park, Ont.*

Diphyllobothrium, and microsporidians. The sampling included 34 lakes from five different river drainages. Further field work would probably disclose additional species of parasites.

Methods

The fish were largely obtained from anglers through the Creel census organization of the Ontario Fisheries Research Laboratory; the viscera were thereby available for examination, the flesh being retained by the anglers. Some trout were caught in nets or by trolling. Before the ice left the lakes fishing was done with lines left set with minnows for bait.

The intestinal parasites were collected by shaking the slit-open intestine in a quart sealer with a 0.7% solution of sodium bicarbonate, the sediment was allowed to settle, and the supernatant was poured off. Following several such washings the sediment was poured into a flat black dish. Few or no parasites were lost in the supernatant. The pieces of intestine were removed from the mixture when it was first shaken up and before it was allowed to settle. Any parasites adhering to the intestine were picked off.

In the black dish, 8 in. square, the trematodes, nematodes, and acanthocephala, all of which were scarce, were removed and their numbers estimated before the usually numerous cestodes that remained were counted. If the cestodes numbered over about 400, a wire frame was put in the dish marking out quadrants; the total number of cestodes was estimated from the number counted in one quadrant, the worms being approximately evenly distributed in the dish.

Cestodes and trematodes were fixed in hot Bouin's fluid, or occasionally in 10% formalin, the former giving the better results. Nematodes were fixed in hot 70% alcohol, and acanthocephala, after their beaks were extruded in lake water, were usually fixed in Bouin's fluid. Parasitic copepods were killed in cold 75% alcohol. All parasites, from which the Bouin's fluid was washed out in several changes of 50% alcohol, were kept in 75% alcohol.

Cestodes and acanthocephala were stained in Delafield's haematoxylin with differentiation in acid alcohol and the trematodes were stained in alum carmine. Nematodes, some acanthocephala, and the copepods were examined unstained, the two former in glycerine or lactic acid. Several cestodes were sectioned to elucidate characters required for identification. Many immature cestodes were identifiable to genus only and have been classified in the table with the species of adult tapeworms.

Annotated List

Protozoa

These microsporidian parasites, forming white cysts in the kidneys, were abundant in both lake and speckled trout from all lakes that were adequately sampled.

There were, however, differences in abundance. The lake trout in Redrock Lake had an average of 1.6 cysts each, in Opeongo 2.7, in Louisa 4.9, and in Butt Lake 8.1, and the speckled trout in Redrock Lake had an average of 3.1

cysts each. The Chi square test of goodness of fit applied to these series demonstrated that the apparent differences between them are all statistically valid. That is, the abundance of these cysts was different in four lakes, and within one lake, speckled trout and lake trout had different numbers of cysts. The reasons for such differences in population are not known.

The cysts were restricted to a short zone in the kidneys about opposite the anterior end of the dorsal fin. Once only was a cyst found further back in the kidney.

Trematoda

Crepidostomum farionis (O. F. Müller, 1784)

This trematode occurred in small numbers in the intestines of a few trout, both lake and speckled, from Happyisle and Redrock Lakes. An unusual occurrence was the presence of two flukes, *Crepidostomum*, in the gall bladder of a speckled trout from Redrock Lake. These flukes were identified by Mr. Allen McIntosh of the Bureau of Animal Industry, Washington, D.C., as *C. farionis*, and it is of interest to note that *C. cooperi* has been recorded from speckled trout in Lake Commandant, Que. (5).

Clinostomum complanatum (Rudolphi, 1814)

One specimen was present in the intestine of a speckled trout from Redrock Lake, probably *en passant* from a perch eaten by the trout.

Azygia angusticauda (Stafford, 1904)

A lake trout from Whitefish Lake had two specimens in its intestine.

Black Spot

One or two small black hard cysts were found in the pectoral fins of three speckled trout out of the 22 examined from Proulx Lake and in eight out of 18 speckled trout from Redrock Lake. None were found in the lake trout. The cysts contain a larval fluke, probably *Apophallus*, but the specimens have not yet been identified.

Neascus sp.

The minute black cysts of this larval parasite of perch were commonly liberated into the intestine of trout in the course of digestion of perch, but they passed through without opening, and are not to be considered intestinal parasites of trout.

Cestoda

Diphyllobothrium sp., possibly *cordiceps* (Leidy, 1871)

Plerocercoids of the genus *Diphyllobothrium* occurred in lake trout, but not in the speckled trout examined, in cysts in the wall of the stomach and adjacent tissues, tending to concentrate in the lesser curvature.

The infection was found abundantly in the following lakes: Big Crooked, Canisbay, Head, Louisa, Source, McKaskill, Butt, and Redrock. The degree of infestation for Louisa Lake averaged 28 plerocercoids per fish, whereas

the average in Butt Lake was only 15, and the difference was statistically significant. Infection was definitely scarce in the following lakes: Smoke, Opeongo, Big Trout, Cache, and Hogan. In Lake Opeongo the plerocercoids were entirely absent from all 89 lake trout examined.

Dr. E. Kuitunen-Ekbaum (2) reported the absence of *Diphyllobothrium* in 18 lake trout from Lake Opeongo in 1937, which is the same condition encountered in the present survey two years later. The great differences in abundance in various lakes are surprising in view of the probability that the parasite matures in fish-eating birds, such as gulls. It may be significant that trout are largely plankton feeders in the lakes in which *Diphyllobothrium* was abundant, as a plankton organism serves as host for the first stage larva.

The two infected lake trout from McKaskill Lake were caught under winter conditions before the ice left the lake and the *Diphyllobothrium* plerocercoids were all dead and disintegrating. This suggests that the infection is lost during the winter and picked up afresh in the spring; however the results from two fish only may be misleading. It happened that heavily infected lakes were not sampled in the spring, so that acquisition of the parasites was not traced, in fact they were not found alive until June. The numbers of cysts observed from June to September showed no significant change; for instance, in Louisa Lake the averages for July, August, and September were 24, 28, and 27.

The plerocercoid stages of several species of *Diphyllobothrium* are apparently indistinguishable morphologically and, as no life history studies were done, the form in Algonquin Park has not been positively identified. It is possibly *D. cordiceps* (Leidy, 1871), which has been reported in *Salmo salar sebago* and found to reach maturity in gulls and pelicans (4, 10, and 13).

That these *Diphyllobothrium* larvae are not *D. latum* (L., 1735) the broad tapeworm of man, is suggested by the fact that the Algonquin forms are encysted in the viscera and not in the flesh while *D. latum* occurs more usually free in the muscles of the host fish (12). Furthermore the experiments by Kuitunen-Ekbaum (2) and those of Woodbury (13) (reported by Magath (8)) suggest that *D. cordiceps* does not develop in man, cat, or dog.

Eubothrium salvelini (Schrank, 1790)

This was the common large tapeworm, occurring in 85% of the lake trout and in 66% of the speckled trout. *Eubothrium* was not found in either of the two fish from Smoke Lake, nor was it found in the seven Dickson Lake fish that were taken in winter. It was present in all other lakes examined. The percentage incidence, average numbers per infected fish, and the frequency distribution in Lake Opeongo are available at the Royal Ontario Museum of Zoology.

Of the 17 lake and speckled trout taken before the ice left the lakes 11 had no tapeworms, five had a few mature *Eubothrium*, and the last fish taken just before the break-up of the ice had mature and immature worms of both this species and *Proteocephalus*. The lakes sampled in late winter were Opeongo,

Proulx, Dickson, and McKaskill. The average numbers of mature *Eubothrium* in lake trout from Lake Opeongo for May, June, and July, were 2, 13, and 14. The numbers of immatures for the same months were 21, 38, and 37.

The Algonquin Park material showed a wide variation in taxonomic characters, which is described elsewhere (7).

Proteocephalus parallacticus MacLulich, 1943

This small tapeworm was abundant in the intestines of the majority of the lake trout from all river drainages. The one significant exception was Redrock Lake from which four lake trout and five speckled trout were examined without finding any *Proteocephalus*. The two speckled trout from Casey Lake also lacked this species. Other speckled trout and a brown trout carried this tapeworm.

Proteocephalus was not in the fish taken in winter except in the last fish taken as the ice began to break up. The average numbers of mature worms in lake trout in Lake Opeongo for May, June, and July were: 16, 91, and 143; the numbers of immatures were: 173, 260, and 459. The frequency distribution in Lake Opeongo is on file at the Royal Ontario Museum of Zoology.

As the combination of characters exhibited by the Algonquin Park material was not described in the literature this was made a new species (6).

Proteocephalus pusillus (Ward, 1908)

Dr. R. V. Bangham found this species in lake trout from Canisbay Lake.

Proteocephalus ambloplitis (Leidy, 1887)

This parasite was probably introduced to this area with the small-mouthed black bass (*Micropterus dolomiu*) that were planted in the southern part of the Park as the definitive hosts are known to be various species of bass (3). The "larvae" or immature worms are commonly found in a number of fish in which they are unable to reach maturity (11). Since trout frequent deeper water than bass during much of the year it is not surprising that the larvae of this bass parasite are of rare occurrence in trout. However a speckled trout from Lake Opeongo had one immature in the stomach, 12 in the caecal part of the intestine, and none in the lower part of the intestine. The food in the stomach, namely, a short-tailed shrew, *Blarina brevicauda*, and a young perch, *Perca flavescens*, suggested that this fish had been feeding near shore where it would be more likely to pick up *P. ambloplitis*. The largest specimen was 4 mm. long. Immature specimens of *P. ambloplitis* were found in the stomach or intestine of three lake trout from Lake Opeongo.

Nematoda

Cystidicola stigmatura (Leidy, 1886)

These roundworms occurred in the air bladders of lake trout and speckled trout in Hogan Lake and Wilkes (formerly Manitou) Lake. The infestations were severe but in most cases appeared to cause no gross damage. Hemorrhage from the bladder wall occurred in two out of the 25 infected trout,

but it is not known whether the worms were responsible for this condition or not. One immature specimen was found in the intestine of a lake trout from Big Trout Lake.

Philonema sp.

These large filarioid worms occurred infrequently free in the body cavity of lake trout from Lake Opeongo. They were not found in fish from any other water.

Large irregular cysts containing dead worms or brown remains of them were apparently due to a foreign-body reaction of the host, walling off the worm after its death. Of 73 trout from Lake Opeongo four had living worms, two of these had encysted dead worms, and 15 others had dead cysts alone. Two fish, not used in the above figures since they were chosen for examination because they had severe infections, had 19 living worms in one and seven living and 14 dead worms in the other fish.

Larval Nematodes not Identified

Delicate larval roundworms were encysted in the viscera, most of them in the liver, of both lake and speckled trout. Other immature roundworms occurred free in the intestines.

Acanthocephala

Leptorhynchoides thecatus (Linton, 1891)

Thorny-headed worms were restricted to lake trout from Lake Opeongo and were not common there.

Parasitic Copepods

Salmincola siscowet (Smith, 1874)

Small numbers of these external parasites occurred occasionally on the skin and fins of lake trout. They were particularly abundant in Louisa and Butt Lakes, and their frequency distributions in those two lakes showed no significant difference. The average number of copepods per fish in those lakes was 1.1.

The type of frequency distribution of a parasite population must be influenced by, and be the result of, the life history of the parasite and the life and ecology of the hosts and environment. For instance, if the parasites were *randomly* distributed to the fish as a result of the ecological situation, the frequency of occurrence of various numbers of parasites per host would follow a Poisson probability series. The facts are, see Table I, Columns 3 and 4, that the copepod numbers show too many high values and are not statistically of the Poisson type. This is true for all the parasites studied in this investigation (microsporidians, *Diphyllobothrium*, *Eubothrium*, *Proteocephalus*, and copepods) with the exception of the microsporidians that occurred in lake trout in Redrock Lake, which is probably a fortuitous circumstance.

In an attempt to throw some light on the factors controlling copepod distribution various mathematical distributions are presented in Table I with

their "Goodness of Fit" indicated for each. It is evident that only the last two distributions fit the facts.

TABLE I

PARASITIC COPEPODS ON LAKE TROUT IN LOUISA AND BUTT LAKES. COMPARISON OF ACTUAL DISTRIBUTION WITH VARIOUS MATHEMATICAL DISTRIBUTIONS*

Number of parasites on a fish	Frequency, numbers of fish		Poisson distribution	"Original" distribution	Neyman's contagious distribution
	Observed data	Smoothed data			
0	68	68	44.5	68.9	72.0
1	29	29	48.5	24.9	21.0
2	14	15	26.4	18.2	17.0
3	11	9	9.5	10.4	10.4
4	2	4	2.6	5.4	5.7
5	4	3	0.6	2.7	3.0
6	2	2	0.1	0.8	1.5
7	2	2	0.0	0.5	1.4
Total number of fish	132	132	132.2	131.9	132.0
Averages, copepods per fish			1.09	1.09	1.09
Probability of a worse fit			0.04	0.43	0.38
Goodness of fit			Nil	Good	Good

* *Note that a probability of 0.50 means that the hypothesis fits perfectly and that a probability of 0.04 means that only four times out of every 100 sets of data could a worse fit be expected, i.e. it is strongly indicated that such a hypothesis fails to account for the facts. The probability is derived from the table of* χ^2 *(1).*

The second last is an original distribution involving two ideas or symbols, one for the average number of swarms of copepods met by a fish, and a second symbol for the average number of parasites picked up by a fish in passing through a swarm. Two assumptions are involved: one, the fish meet the swarms purely by chance, and, two, the numbers of parasites picked up in passing through swarms form a random distribution. Both assumptions, therefore, are described by Poisson series. The net result is a distribution that allows for the swarming of the nauplii and the two above assumptions of pure chance. The excellent "Goodness of Fit" is strong evidence that the above hypotheses are a first approximation to the truth. The numerical values are as follows: mean number of swarms met by a fish per season equals 0.98; and mean number of copepods picked up in passing through one swarm equals 1.11.

The third, "Neyman's Contagious Distribution of Type *A* with two Parameters" (9), is based on the idea of grouping or "contagiousness" i.e. if there is any infestation at all several parasites are apt to be present. It is plain that a population built up on the principles described in the previous paragraph will display a degree of this quality of "contagiousness".

Salmincola edwardsii (Olsson 1869)

This species was carried in small numbers by speckled trout. In one case only was the infestation severe; this was in a speckled trout from Dickson Lake with 21 copepods on the gills and one on the adipose fin on March 20.

Acknowledgments

Acknowledgments are due to the Superintendent, Mr. F. A. MacDougall, for making the investigation possible by loaning the writer (who was on the ranger staff of the Park) to the Ontario Fisheries Research Laboratory and providing a canoe and other facilities. The Director of the above laboratory, Professor W. J. K. Harkness, and other members of the staff, aided materially with laboratory space, supplies, and co-operation in many ways. Much of the laboratory work was done in the Ontario Research Foundation, Toronto, and grateful acknowledgment is made to the Director of the Foundation, Dr. H. B. Speakman, and to Dr. S. Hadwen, the Director of Pathology and Bacteriology, and his assistant, Dr. A. M. Fallis. The Director of the Royal Ontario Museum of Zoology, Professor J. R. Dymond, gave work space in the Museum for part of the time.

Aid of various sorts from the following persons is also acknowledged: Dr. R. V. Bangham of the College of Wooster, Wooster, Ohio, Mr. J. S. Hart of the Ontario Fisheries Research Laboratory, and Dr. E. W. Price, Bureau of Animal Industry, Washington, D.C.

References

1. FISHER, R. A. Statistical methods for research workers. 6th ed. rev. and enl. Oliver and Boyd, Edinburgh and London. 1936.
2. KUITUNEN-EKBAUM, E. *Diphyllobothrium* sp. plerocercoid in the lake trout from Algonquin Park, Ontario. Manuscript. 1937.
3. LA RUE, G. R. A revision of the cestode family Proteocephalidae. Illinois Biol. Monogr. 1 : 1-351. 1914.
4. LINTON, E. A contribution to the life history of *Dibothrium cordiceps*, a parasite infecting the trout of Yellowstone lake. Bull. U.S. Fish Commission, 9 : 337-358. 1891.
5. LYSTER, L. L. Parasites of freshwater fish. II. Parasites of speckled trout and lake trout and the fish found associated with them in Lake Commandant, Que. Can. J. Research, D, 18 : 66-78. 1940.
6. MACLULICH, D. A. *Proteocephalus parallacticus*, a new species of tapeworm from lake trout, *Cristivomer namaycush*. Can. J. Research, D, 21 : 145-149. 1943.
7. MACLULICH, D. A. Morphological variation in the cestode *Eubothrium salvelini* (Schrank 1790). Manuscript.
8. MAGATH, T. B. The relation of *Diphyllobothrium latum* infestation to the public health J. Am. Med. Assoc. 101 : 337-341. 1933.
9. NEYMAN, J. On a new class of "contagious" distributions, applicable in entomology and bacteriology. Ann. Math. Stat. 10 : 35-57. 1939.
10. THOMAS, L. J. Further studies on the life cycle of a cestode from the herring gull. J. Parasitol. 25 : 20. 1939.
11. VAN CLEAVE, H. J. and MUELLER, J. F. Parasites of Oneida lake fishes. Part III. A biological and ecological survey of the worm parasites. Roosevelt Wild Life Ann. 3 : 161-334. 1934.
12. WARDLE, R. A. Fish-tapeworm, Biol. Board Can. Bull. 45. 1935.
13. WOODBURY, L. A. Infectivity of the plerocercoids of *Diphyllobothrium cordiceps* (Leidy) for man. J. Parasitol. 21 : 315-316. 1935.

STUDIES ON TRICHINOSIS

IV. HUMAN INCIDENCE IN MONTREAL[1]

By T. W. M. Cameron[2]

Abstract

On the basis of 539 unselected samples examined, the incidence of human trichinosis in the Montreal district was found to be 1.5%.

From December, 1940, until February, 1943, samples of diaphragm were collected at the morgue of the Royal Victoria Hospital, Montreal, Que., and from June, 1941, until February, 1943, at the Montreal General Hospital as well. Except that children under one year old were excluded on the hypothesis that they could only have been exposed to infection prenatally, no selection was made. All samples were taken from the diaphragm and conveyed in waterproof cardboard containers to this Institute where they were examined both by compression and pepsin digestion techniques for the presence of encysted trichina larvae.

Three hundred and one diaphragms were examined from the Royal Victoria Hospital and 238 from the Montreal General Hospital—a total of 539 in all. Of these, eight, or 1.5%, were positive (five from the Royal Victoria Hospital and three from the Montreal General Hospital). All the infections were light, the heaviest being 19.5 larvae per gram of diaphragm. In all samples the larvae were alive; no dead larvae or trichina-like objects were detected by examination with the compressorium. These results agree with those published by E. Kuitunen-Ekbaum at Toronto (2) while the Montreal survey was in progress. She examined 420 cases in all, but 77 were under one year old. There were accordingly 343 cases comparable with the Montreal series. Of these, six were positive, although in three of them, the cysts were calcified. This gives an incidence of 1.75%. The infections were very light ones.

It seems legitimate to conclude, therefore, that the incidence of human trichinosis in Eastern Canada is probably about 1.5 to 1.75%. It must be recalled, however, that this is a helminthic infection, which is not contagious, and that incidence figures do not have the same significance as in bacterial diseases. A single infected pig may cause a sharp local outbreak with serious disease, even although the incidence for the country as a whole is low. A more pertinent figure is the incidence in hogs, although even then the figures require interpretation in the light of the feeding habits of the people. The light infections in the Montreal series suggest that such infections as do occur, do so as the result of eating undercooked, rather than uncooked pork. Nevertheless, outbreaks due to uncooked pork have been recorded in Montreal.

[1] *Manuscript received September 30, 1943.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Professor, McGill University; and Director, Institute of Parasitology, Macdonald College, Que., Canada.*

In Eastern Canada, the general incidence of hog trichinosis is 0.57% (1). In the United States, the hog incidence is higher, 2.5%, and the human incidence much higher, 16.7% (3). It will be noted, however, that the incidence of hog to human trichinosis in Canada is about 1 : 3, while in the United States, the ratio is 1 : 6.68; that is, one hog infects about twice as many people in the United States as a hog in Canada.

The larger amount of hog trichinosis in the United States is due to some hogs being fed uncooked garbage; the incidence in those fed no garbage, or cooked garbage, is comparable with the Canadian figure. As the exact proportions of these classes is unknown, the figures for the United States are not absolutely accurate and the incidence in hogs may be as low as 1.5%. This merely increases the disproportion. This can be explained only by assuming that a higher proportion of persons in the United States eat pork undercooked or uncooked than in Eastern Canada.

The elimination of feeding uncooked garbage might be expected to reduce human infection in the United States to between 3 and 4%, assuming no change in the feeding habits of the people. Obviously, therefore, education on the dangers of raw or undercooked pork would be still essential.

It also follows from these comparative figures that the elimination of uncooked garbage from the hogs' diet will reduce the incidence and especially the severity of infection, but it will not eliminate it. This may be due partly to evasion of the law or it may be due to some cause still unknown.

The collection and digestion of the specimens was carried out by Sgt. Douglas Conlon, R.C.A.F., and it was made possible through the co-operation of Professors G. Lyman Duff, L. J. Rhea, and Theo. R. Waugh.

References

1. CAMERON, T. W. M. Can. J. Research, D, 18 : 83-85. 1940.
2. KUITUNEN-EKBAUM, E. Can. Pub. Health J. 32 : 569-573. 1941.
3. WRIGHT, W. H. *In* Report of the New York State Trichinosis Commission. Legislative Document, No. 35, p. 26. 1942.

SECTION D

INDEX TO VOLUME 21

Authors

Allen, D. E.—See Collier, H. B.

Anderson, R. M. and Rand, A. L.—Variation in the porcupine (genus *Erethizon*) in Canada, 292.

Armstrong, F. H.—See Hayes, F. R.

Arnason, A. P.—See Glen, R.

Atwood, C. E. and Peck, O.—Some native sawflies of the genus *Neodiprion* attacking pines in Eastern Canada, 109.

Buckby, L.—See Reedman, E. J.

Cameron, T. W. M.—Studies on trichinosis. IV. Human incidence in Montreal, 413.

Charnley, F. and Young, O. C.—The effect of freezing and thawing on the quality of canned herring, 8.

Collier, H. B., Allen, D. E., and Swales, W. E.—Observations on the fate of phenothiazine in domestic animals, 151.

Cowan, I. McT.—Notes on the life history and morphology of *Cephenemyia jellisoni* Townsend and *Lipoptena depressa* Say, two dipterous parasites of the Columbian black-tailed deer (*Odocoileus hemionus columbianus* (Richardson)), 171.

Ettinger, G. H.—See Sawyer, M. E. M.

Fulton, C. O.—See Gibbons, N. E.

Gibbons, N. E.—See Thistle, M. W.

Gibbons, N. E. and Fulton, C. O.—Dried whole egg powder. VII. Effect of temperature and moisture on the bacterial content of liquid and dried egg, 332.

Glen, R., King, K. M., and Arnason, A. P.—The identification of wireworms of economic importance in Canada, 358.

Glenny, F. H.—Main arteries in the neck and thorax of the rhea embryo, 189.

Grant, G. A.—See White, W. H.

Hart, J. S.—The cardiac output of four freshwater fish, 77.

Hayes, F. R. and Armstrong, F. H.—Growth of the salmon embryo, 19.

King, K. M.—See Glen, R.

MacLulich, D. A.—Parasites of trout in Algonquin Provincial Park, Ontario, 405.

Proteocephalus parallacticus, a new species of tapeworm from lake trout, *Cristivomer namaycush*, 145.

Miller, R. B.—Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta.

I. Introduction and the life of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas) in the definitive host, *Esox lucius*, 160.

II. The eggs, coracidia, and life in the first intermediate host of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas), 284.

Morrison, F. O.—The standardizing of a laboratory method for comparing the toxicity of contact insecticides, 35.

Munro, J. A.—Studies of waterfowl in British Columbia. Mallard, 223.

Pearce, J. A.—Behaviour and nature of the fluorescing substances in dried egg powders, 98.

The dehydration of pork, 394.

Pearce, J. A.—See Thistle, M. W.

Pearce, J. A., Thistle, M. W., and Reid, M.—Dried whole egg powder. VIII. An improved fluorescence method and some factors affecting the measurement, 341.

Peck, O.—See Atwood, C. E.

Rand, A. L.—See Anderson, R. M.

Reedman, E. J.—The production of canned precooked chicken, 324.

Reedman, E. J. and Buckby, L.—Investigations on the use of Irish moss in canning of meat, 348.

The vitamin B_1 content of canned pork, 261.

Reid, M.—See Pearce, J. A., Thistle, M. W., White, W. H., and Woodcock, A. H.

Sawyer, M. E. M. and Ettinger, G. H.—The cardiac action of posterior pituitary extract in physiological doses, in the normal dog, and after partial and complete denervation of the heart, 311.

Steinberg, A. G.—The development of the wild type and Bar eyes of *Drosophila melanogaster*, 277.

Swales, W. E.—See Collier, H. B.

Thistle, M. W.—See Pearce, J. A. and White, W. H.

Thistle, M. W., Pearce, J. A., and Gibbons, N. E.—Dried whole egg powder. I. Methods of assessing quality, 1.

Thistle, M. W., Reid, M., and Gibbons, N. E.—Dried whole egg powder. V. Definition and properties of low grade egg powders, 267.

White, W. H.—See Woodcock, A. H.

White, W. H. and Grant, G. A.—Dried whole egg powder. III. A refractometric method for the determination of solubility, 203.

White, W. H. and Thistle, M. W.—Dried whole egg powder.

II. Effect of heat treatment on quality, 194.

IV. Effect of moisture content on keeping quality, 211.

White, W. H., Thistle, M. W., and Reid, M.—Dried whole egg powder. VI. Effect of storage temperature and gas packing on keeping quality, 271.

Woodcock, A. H.—Colour of meat. IV. Measurement of the colour of bacon, 90.

Woodcock, A. H. and Reid, M.—Dried whole egg powder. IX. Effect of drying conditions on quality, 389.

Woodcock, A. H. and White, W. H.—Canadian Wiltshire bacon. XXIII. The effect of concentration of curing salts on colour and colour stability, 85.

Young, O. C.—See Charnley, F.

SECTION D

INDEX TO VOLUME 21

Subjects

Acanthocephala, See under Parasites.

Agar
Irish moss as substitute for, in canning of chicken, 348.
used in canning chicken, 328.

Algae, See Chondrus crispus.

Air, Effect of, on storage of dried egg powder, 275.

Ameiurus nebulosus, See under Fish.

Amia calva, See under Fish.

Anas platyrhynchos, See under Birds.

Animals, See Deer, Dog, Horse, Pig, Porcupine, Rabbit, and Sheep.

Arteries, in the neck and thorax of the rhea embryo, 189.

Atropine, Effect of, on response of dog heart to posterior pituitary extract, 314–322.

Aves, See Birds.

Bacon, See under Meat.

Bacteria
in dried egg powder
Formation of fluorescing substances as affected by, 106.
Numbers of, as affected by moisture, 337.
Numbers of, as affected by temperature, 334.
Numbers of, as test of quality, 2–6.
in liquid egg, Numbers of, as affected by temperature, 333.

Bar eyes of Drosophila melanogaster, Development of, 280.

Beating value as test of quality in dried egg powder, 2–6.

Birds
Anas platyrhynchos in British Columbia
Distribution and seasonal movements of, 224.
Economic status of, 258.
Factors restricting numbers of, 245.

Birds—*concluded*
Anas platyrhynchos in British Columbia—*concluded*
Food and feeding habits of, 247, 251.
Reproduction of, 238.
Sex ratio of, 247.
Rhea, Arteries in the neck and thorax of embryo of, 189.

Blood
Output of, from heart of four fish species, 77.
pressure, See Heart, Dog.
system, See Arteries.

Bowfin, See Fish, Amia calva.

Brown trout, See Fish, Salmo fario.

Canning
of chicken
Device for testing jelly strength used in, 329.
Factors affecting quality in, 325.
Methods of precooking in, 325.
Use of Irish moss in, 348.
of herring, Effect of freezing and thawing on, 8.

Carbon dioxide
Blood of fish and its relation to stroke output of the heart as affected by, 81.
Storage of dried eggs as affected by packing in, 275.

Carp, See Fish, Cyprinus carpio.

Catfish, See Fish, Ameiurus nebulosus.

Catostomus commersonii, See under Fish.

Cephenemyia jellisoni, See under Insects.

Cestodes, See under Parasites.

Chicken, See under Meat.

Chondrus crispus, Use of, in canning chicken, 348.

Cladocerans as possible hosts of Triaenophorus, 288.

Colour of bacon
Effect of concentration of curing salts on, 85.
Measurement of, 90.

Colour stability of Canadian Wiltshire bacon, Effect of concentration of curing salts on, 85.

Copepods, See under Parasites.

Coracidia of Triaenophorus crassus and T. nodulosus in the first intermediate host, 286.

Coregonus clupeaformis, See under Fish.

Cristivomer namaycush, See under Fish.

Curing pickle, Effect of concentration of salts in, on colour and colour stability of Canadian Wiltshire bacon, 85.

Curing salts, See under Salts.

Cyclops, as host for Triaenophorus species, 164, 287, 288.

Cyprinus carpio, See under Fish.

Deer, Black-tailed, Life history and morphology of two dipterous parasites of, 171, 180.

Deer louse fly, See Insects, Lipoptena depressa.

Deer nostril fly, See Insects, Cephenemyia jellisoni.

Deer tick, See Insects, Lipoptena depressa.

Dehydration
of pork, 394.
See also Drying.

Diaptomus ashlandi, Infection of, with Triaenophorus, 287.

Diptera, See Insects for members of.

Dog(s)
Fate of phenothiazine in, 157.
Normal and after partial and complete denervation of the heart, Cardiac action of posterior pituitary extract in, 311.

Domestic animals, See Animals.

Dried egg powder, See Egg powder.

Drosophila melanogaster, See under Insects.

Drying, See Dehydration and Egg powder, Dried.

Duck, Mallard, See Birds, Anas platyrhynchos.

Egg powder, Dried
Bacteria in
and their effect on formation of fluorescing substances, 106.
as affected by moisture, 337.
as affected by temperature, 334.
as test of quality, 2–6.
Flavour quality of
An improved fluorescence method for measuring, 341.
Some factors affecting the measurement of, 341.
Fluorescence of
as test of quality, 3–6.
Behaviour and nature of substances causing, 98.
Effect of enzymes and bacteria on substances causing, 106.
Effect of heat on formation of substances causing, 105.
Effect of pH on, 345.
Effect of protein solvents and precipitants on, 102.
Effect of temperature on, 345.
Fat solvents and their effect on, 99.
Fractions causing, 101.
Quality as assessed by, 98.
Source of materials causing, 98.
to assess quality, An improved method for using, 341.
Keeping quality of
Effect of gas packing on, 271.
Effect of moisture content on, 211.
Effect of storage temperature on, 211, 271.
Effect of tablet method of storing on, 275.
Low grade, Definition and properties of, 267.
Quality of
as affected by drying conditions, 389.
as affected by heat treatments, 194.
as tested by numbers of bacteria, 2–6.
Methods of assessing, 1.
See also Egg powder, Dried (Flavour and Keeping).
Solubility of, Refractometric method for determining, 203.
Storage of
and the effect of temperature on bacterial content, 336.
as affected by temperature and gas packing, 275.
by tablet method and its effect on keeping quality, 275.

Egg(s)
Liquid, Effect of temperature on bacterial content of, 333.
of Triaenophorus in the first intermediate host, 284.
White of, dried, Fluorescence in, 99.
Yolk of, dried, Fluorescence in, 99.

Embryo
of rhea, Main arteries in neck and thorax of, 189.
of salmon, Growth of, 19.

Enzymes, Effect of, on formation of fluorescing substances in dried eggs, 106.

Erethizon dorsatum in Canada
Distribution of, 293.
Form of, 293.
Variations in pelage and skull characters of, 296.

Esox lucius, See under Fish.

Fat extraction, Fluorescence values of dried egg powder as affected by method of, 341.

Filter method of measuring colour of meat, 92.

Fish
Ameiurus nebulosus, Cardiac output of, 77.
Amia calva, Cardiac output of, 77.
blood, Output of, from heart, 77.
Cardiac output of four species of, 77.
Catostomus commersonii, Cardiac output of, 77.
Coregonus clupeaformis, host for plerocercoids of Triaenophorus, 161.
Cristivomer namaycush
A new tapeworm species from, 145, 148.
Parasites of, in Algonquin Park, 405.
Cyprinus carpio, Cardiac output of, 77.
Esox lucius, Life history of Triaenophorus crassus and T. nodulosus in, 160, 164.
heart, Stroke output of, 77.
Herring, Effect of freezing and thawing on quality of, 8.
Leucichthys tullibee, host for plerocercoids of Triaenophorus, 161.
Lota maculosa, host for plerocercoids of Triaenophorus, 164.
of Lesser Slave Lake, Alberta, Cestodes of, 160.
Percopsis omiscomaycus, host for plerocercoids of Triaenophorus, 164.
Prosopium sp., host for plerocercoids of Triaenophorus, 161.
Salmo fario
A new tapeworm species from, 145, 148.
Parasites of, in Algonquin Park, 405.

Fish—*concluded*
Salmo salar, Growth of embryo of, 19.
Salvelinus fontinalis
A new tapeworm species from, 145, 148.
Parasites of, in Algonquin Park, 405.
Species of, as hosts for various stages of three Triaenophorus species, 161, 164.
Stizostedion vitreum, host for an apparently undescribed species of Triaenophorus, 164.

Flavour of dried egg powder, An improved fluorescence method and some factors affecting the measurement of, 341.

Flies, See Diptera.

Flukes, See Parasites, Trematodes.

Fluorescence, See under Egg powder.

Freezing, Effect of, on quality of canned herring, 8.

Fungi, See Bacteria.

Gas packing of dried egg powder, Effect of, on keeping quality, 275.

Gelatine used in canning chicken, 328.

Growth of the salmon embryo, 19.

Heart
of dog
after bilateral vagotomy, Effect of posterior pituitary extract on, 319.
after complete denervation, Effect of posterior pituitary extract on, 321.
after thoracic sympathectomy, Effect of posterior pituitary extract on, 319.
after unilateral vagotomy and bilateral sympathectomy, Effect of posterior pituitary extract on, 321.
Normal, Effect of posterior pituitary extract on, 313.
of fish, Stroke output of, 77.
rate, See Heart of dog and fish.

Heat, See Temperature.

Herring, See under Fish.

Horses, Fate of phenothiazine in, 156.

Immersion technique, used in comparing toxicity of insecticides, 37, 39.

Insecticides, Standardizing of a laboratory method for comparing the toxicity of, 35.

Insects
Cephenemyia jellisoni, Life history and morphology of, 171.

Insects—*concluded*
Drosophila melanogaster
as test animal in comparing toxicity of insecticides, 36.
Development of wild type and Bar eyes of, 279, 280.
Eversion of the cephalic complex of, 281.
Lipoptena depressa, Life history and morphology of, 180.
Neodiprion
flemingi, n. sp. Peck, Description of adults and larvae of, 121, 132.
lanielensis, n. sp. Peck, Description of adults and larvae of, 115, 131.
species attacking pines in Eastern Canada, Identification of, 110, 123.

Irish moss, Use of, in canning meat, 348.

Jelly
Irish moss used as, in canning of meat, 348.
on canned chicken, Device for testing strength of, 329.

Lake trout, See Fish, Cristivomer namaycush.

Leucichthys tullibee, See under Fish.

Ling, See Fish, Lota maculosa.

Lipoptena depressa, See under Insects.

Lota maculosa, See under Fish.

Louse fly, Deer, See Insects, Lipoptena depressa.

Mallard, See Birds, Anas platyrhynchos.

Meat
Bacon, See Pork.
Canadian Spiced Ham, See Pork, Raw and canned.
Canadian Spiced Pork, See Pork, Raw and canned.
Chicken, Canning of
Device for testing jelly strength used in, 329.
Factors affecting quality in, 325.
Irish moss used as jelly in, 348.
Methods of precooking in, 325.
Herring, Canned, Effect of freezing and thawing on quality of, 8.
Pork
Colour and colour stability of, as affected by curing salts, 85.
Dehydration of, 394.
Measurement of the colour of, 90.

Meat—*concluded*
Pork—*concluded*
Method of determining vitamin B_1 in, 262, 263.
Raw and canned, Vitamin B_1 content of, 262, 264.
Trichinosis in Montreal, 413.
Spiced luncheon, See Pork, Raw and canned.

Moisture in dried egg powder, See Egg powder, Dried.

Moss, Irish, See Irish moss.

Neodiprion, See under Insects.

Nicotine alkaloid, used in tests for comparing toxicity of insecticides, 38, 39.

Nicotine sulphate, used in tests for comparing toxicity of insecticides, 38, 39.

Nitrogen, Storage of dried eggs packed in, 275.

Nostril fly, Deer, See Insects, Cephenemyia jellisoni.

Odocoileus hemionus columbianus, Host for two dipterous parasites, 171, 180.

Oxygen affinity of blood of fish, Relation to stroke output of the heart, 81.

Palatability as test of quality of dried egg powder, 3–6.

Parasites
Acanthocephala parasitic on trout in Algonquin Park, 410.
Cestodes
parasitic on trout in Algonquin Park, 407.
Proteocephalus parallacticus, a new species of, in trout, 145.
Triaenophorus crassus
in the first intermediate host, 284.
Life history of, in pike, 160.
Triaenophorus nodulosus, in the first intermediate host, 284.
Triaenophorus sp.?, Record of observation of, in pickerel, 164.
Copepods
as hosts or probable hosts of Triaenophorus, 287.
parasitic on trout in Algonquin Park, 410.
Insects
Cephenemyia jellisoni, parasitic on deer, 176.
Lipoptena depressa, parasitic on deer, 180.

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012.
This book can be issued on or after _____

Return Date	Return Date